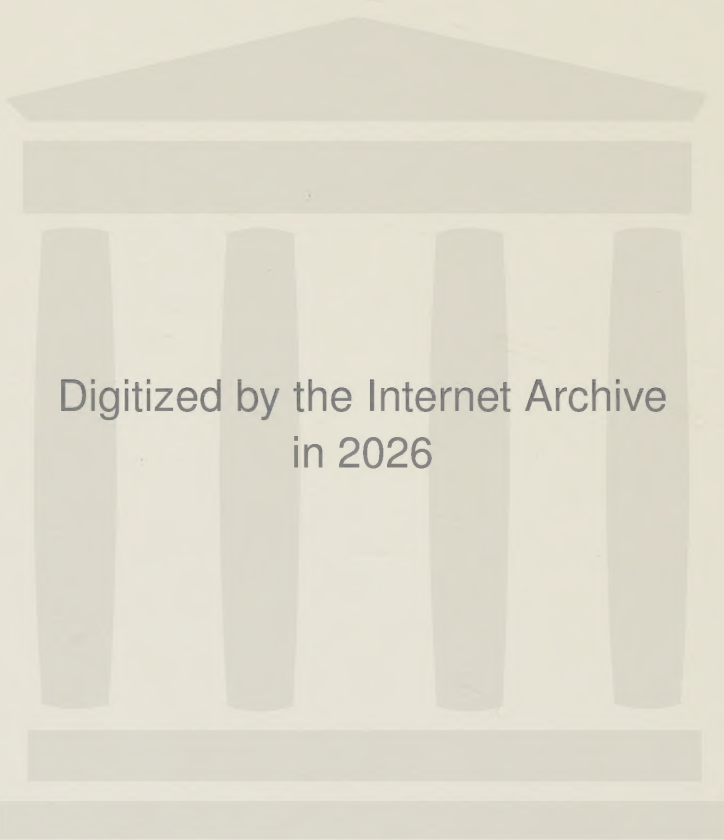


Adrenergic and Peptidergic Neuro-
muscular Mechanisms in the Human
Fallopian Tube, with Special Regard
to Cyclic Influences

Göran Helm

LUND 1981



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This thesis is dedicated to

Vivi-Ann

Anna Emma Ida



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The thesis is based on the following papers:

- I Helm, G., Owman, Ch., Rosengren, E., and Sjöberg, N.-O.:
Regional and cyclic variations in norepinephrine concentration
of the human Fallopian tube.
Biol Reprod, in press 1981.
- II Sporrang, B., Helm, G., Owman, Ch., and Sjöberg, N.-O.:
Electron microscopic and pharmacologic evidence for a functional
adrenergic innervation of the smooth musculature in the human
Fallopian tube.
Brain Res Bull, in press 1981.
- III Helm, G., Owman, Ch., Sjöberg, N.-O., and Walles, B.:
Motor activity of the human Fallopian tube in vitro in relation
to plasma levels of estradiol and progesterone and the influence
of noradrenaline.
J Reprod Fertil, in press 1981.
- IV Helm, G., Owman, Ch., Sjöberg, N.-O., and Walles B.:
Quantitative pharmacological characterization of beta-receptors
and two types of alpha-receptors mediating sympathomimetic
smooth muscle response in the human Fallopian tube at various
cyclic stages.
Acta Physiol Scand, in press 1981.
- V Batra, S., Helm, G., Owman, Ch., Sjöberg, N.-O., and Walles, B.:
Female sex steroid concentrations in the ampullary and isthmus
regions of the human Fallopian tube and their relationship to
the plasma concentrations during the menstrual cycle.
Am J Obstet Gynecol 136:986-991, 1980.
- VI Alm, P., Alumets, J., Håkanson, R., Helm, G., Owman, Ch.,
Sjöberg, N.-O., and Sundler, F.:
VIP nerves in the human female genital tract.
Am J Obstet Gynecol 136:349-351, 1980.
- VII Helm, G., Ottesen, B., Fahrenkrug, J., Larsen, J.-J. Owman, Ch.,
Sjöberg, N.-O., Stolberg, B., Sundler, F., and Walles, B.:
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reproductive tract: distribution and motor effects.
Biol Reprod 25:227-234, 1981.
- VIII Helm, G., Håkanson, R., Leander, S., Owman, Ch., Sjöberg, N.-O.,
and Sporrang, B.:
Neurogenic relaxation mediated by vasoactive intestinal polypeptide
(VIP) in the isthmus of the human Fallopian tube.
Regulatory Peptides, in press 1981.

INTRODUCTION

Ovum transport: general aspects

The transport of the ovum and the blastocyst through the human Fallopian tube is a complex process. After follicular rupture the ovum is picked up by the oviductal fimbriated end which is brought in contact with the ovary by means of contraction of the myotube, the smooth muscle of the mesosalpinx as well as the tubo-ovarian ligaments (Westman 1937, Doyle 1954, Blandau 1969). After fast passage of the ovum through the distal ampulla, which takes place in a pattern of to-and-fro movements, the ovum is retained in the proximal part of the ampullary region, near the ampullary-isthmic junction, for approximately 72 hours (Cheviakoff et al. 1976), allowing fertilization to take place. In about 80 hours after ovulation the blastocyst reaches the uterine cavity (Croxatto et al. 1978, Diaz et al. 1980).

Though the pattern and time required for the ovum transport are well established the regulation of the transport process is still largely unknown. Knowledge of these regulatory mechanisms are important, not least in regard to preventing or facilitating fertility (Hafez 1973, Adams 1976, Chang 1976). Several factors may be involved in the ovum transport processes; e.g. the epithelial ciliary activity, oviductal secretions, the muscular contractions of the tubal wall and its nervous supply.

Ciliary activity and oviductal secretion

The ampullary part is more ciliated than the isthmic part, and it is also believed that the ciliary activity is more important in the ampulla than in the isthmus (Blandau and Verdugo 1976). The significant role of tubal cilia in ovum transport through the rabbit ampulla has been elucidated by Eddy et al. (1978). In the human Fallopian tube the relative importance of the ciliation or the secretion (Jansen 1980) from the tubal wall during ovum transport is not well understood.

Smooth musculature

Most of the interest in the field of ovum transport has been focused upon the muscular activity in the myosalpinx. The transient arrest of the ovum at the ampullary-isthmic junction, which seems crucial for successful concep-

tion, may be an effect of a sphincter-like function (Brundin 1965, Pauerstein et al. 1974, Harper et al. 1976).

The muscle components in the human oviduct are arranged in separate layers, with an essentially circular and longitudinal orientation. The musculature of the isthmic part is more prominent than in the ampulla. Three separate layers can be distinguished in the proximal part of the isthmus, but the inner longitudinal arrangement of muscle bundles disappears towards the ampullary-isthmic junction, where only one prominent, circularly oriented and one outer longitudinal layer can be found (Williams 1891, David and Czernobilsky 1968).

Several methods have been used to study the tubal motility with or without correlation to ovum transport. Perfusion of the oviduct with CO₂ or saline solution has been used to obtain knowledge about the flow resistance (Rubin 1947, Brundin 1965, Seitchik et al. 1968, Levy and Lindner 1972). The information from these experiments seems to have limitations. The site of resistance to flow cannot be determined, and also other organ structures can influence the resistance. Moreover, the measurement requires a distending stimulus which may influence the recordings, and insufflation is often done in isthmic-ampullary direction while the ovum moves from ampulla to isthmus. Intratubular pressure recordings have been performed by open ended catheters or microballoon techniques (Spilman and Harper 1974, Maia and Coutinho 1976). This type of intraluminal monitoring also has limitations. Distension and occlusion of the oviduct will result in nonreliable motor activity and the obstruction will prevent the study of normal gamete transport. The regional extension of the contractions is usually impossible to demonstrate with these methods (Blandau et al. 1975, Daniel 1976), and contamination of extra-tubal smooth muscle activity cannot be sorted out.

Transducer systems have been developed to measure the tubal muscular activity extraluminally. Among others, the miniature mercury strain gauges (Maistrello 1971) and ultrasound transducers (Hodgson et al. 1973) have been used in vivo. The integrated circuit technology has led to smaller and highly sensitive transducers with possibility to record mechanical contractions, pressure waves, and electrical waves simultaneously (Nelsen et al. 1976). These techniques, together with electrical recording of tubal smooth muscle activity (Talo 1980), are the most promising in vivo approaches so far. The in vivo methods for registration of tubal muscular

activity have a number of practical and ethical limitations and are difficult to standardize and evaluate appropriately for quantitative pharmacological analysis. Methods in vitro allow more precisely controlled conditions and make pharmacological manipulation possible (Seitchik et al. 1968, Vastik-Fernandez et al. 1975, Moawad et al. 1976, Owman et al. 1976, Gimeno et al. 1978). The influence of surrounding tissues is possible to avoid. On the other hand, the integrated activity of the smooth musculature in the tube and in adjoining peritoneal structures is impossible to evaluate.

Depending on the aim of the study, essentially two types of smooth muscle preparations have been used as in vitro models. One consists of carefully microdissected and prepared smooth muscle elements from the circular and longitudinal musculature (Lindblom et al. 1978, Lindblom 1979). In this way the two major types of smooth musculature become well defined. Another type of preparation, used in the present study, utilizes segments from the entire intact wall of the oviduct, except for its connective tissue. This kind of preparation, with a preserved tubal wall architecture, including close appositions between cells in the various muscle layers, has certain advantages. As a functional unit this preparation will more closely reflect conditions in situ.

Nerve supply

The development of the Falck-Hillarp histofluorescence technique for visualization of adrenergic nerves (Falck et al. 1962), applied together with chemical determinations of noradrenaline and denervation experiments, has led to informative knowledge about the adrenergic innervation in the human female genital tract. It is now a well-known fact that the Fallopian tube has a rich adrenergic supply, partly by so called "short" adrenergic neurons originating from ganglion cells in the paracervical region (Nakanishi et al. 1967, Owman et al. 1967, Sjöberg 1967, Moawad et al. 1977, Sjöberg et al. 1977).

The arrangement of the adrenergic nerve terminals in the myotube suggests an important role in the motor function. In the ampullary part the relatively thin muscle layers contain only a few nerves compared to the isthmus, where the fluorescent adrenergic nerves are numerous and run in the same direction as the smooth muscle cells. This high density in the isthmus

part supports the theory of "isthmus sphincter blockade" of the ovum. In agreement with this, chemical determination of noradrenaline shows a higher level of the adrenergic transmitter in the isthmus than in the ampulla (Owman et al. 1967, Dujovne et al. 1976). Moreover, the system of short adrenergic neurons has a unique sensitivity to the influence of sex hormones (cf. Owman and Sjöberg 1976b, Thorbert 1978).

The knowledge about the cholinergic innervation of the female genital tract is limited, partly due to the lack of useful specific histochemical methods. Acetylcholinesterase activity, though sparse, has been found to occur in the oviduct (Jacobowitz and Koelle 1965, Owman and Sjöberg 1966). Cholinomimetic stimulation of the rabbit oviduct in vitro suggests that an adrenergic link may be involved in the motor effects (Howe 1976). However, the significance of the cholinergic system is not clear.

AIM OF THE INVESTIGATION

The study was performed to obtain basic information about autonomic neuro-effector mechanisms in the human Fallopian tube through an analysis of the two systems of smooth musculature (circular and longitudinal) in the two main regions of the organ (isthmus and ampulla). Isolated preparations were chosen to allow for correlated chemical and quantitative pharmacological studies, and the tubal wall was used intact to maintain the complex cellular architecture which may be of importance for the response pattern of the tissue. The following main problems were investigated:

1. The detailed regional distribution of adrenergic nerves in the human Fallopian tube and evidence that they participate in a smooth muscle control mechanism.
2. Characterization of the motor activity of the tubal smooth musculature, and how this activity is influenced by the sympathetic neurotransmitter.
3. Quantitative pharmacological characterization of the adrenergic receptors mediating the sympathomimetic effects on the tubal smooth musculature.
4. Investigation of any cyclic variability in the function of the tubal adrenergic nerves in the sympathomimetic response pattern and in the properties of the receptors mediating this smooth muscle response.
5. Correlation between the levels of estrogen and progesterone in plasma (which is being used to define cyclic stage) and in tubal tissue, as an approach to interpret any cyclic fluctuations of tubal neuromuscular functions.
6. The role of vasoactive intestinal polypeptide (VIP) in tubal smooth muscle regulation along with the classical adrenergic and cholinergic autonomic mechanisms.

EXPERIMENTAL DESIGN

Human tubal material was obtained from regularly menstruating women undergoing laparotomy. The material also included pregnant patients and patients taking contraceptive pills or in a menopausal stage. Macroscopically abnormal tubes were not used. Tubes from more than 500 women have been examined.

High performance liquid chromatography

Chemical determinations of the noradrenaline content of the Fallopian tube have been carried out by means of high performance liquid chromatography (Paper I). The tubes have been sectioned in isthmus, ampulla, fimbriated end, as well as smaller consecutive parts from isthmus and ampulla for regional analysis. The tissue specimens were homogenized in 0.4 M perchloric acid and the catecholamines adsorbed on alumina. After elution the final chromatographic separation was done on a Nucleosil 5 SA column.

The precision for repetitive determination of dopamine, noradrenaline and adrenaline was evaluated with an internal standard, and was found to be 3% for noradrenaline, 4.6 % for adrenaline, and 4.6 % for dopamine. The plot of peak height versus the amount of injected amines was found to be linear over the concentration range of interest. The detection limit for the catecholamines in the samples was 250 pg.

The results obtained with this high pressure liquid chromatography method were on several occasions compared to those found when a reversed-phase liquid chromatographic system was used. In these cases a column containing Nucleosil C₁₈ was used instead of the cation-exchange resin. The correlation between the two methods was high. The specificity of the methods was further examined by addition of small amounts of authentic material to the sample. No inhomogeneities in the peaks examined could be observed. For further methodological details, see Hansson and Rosengren (1979).

Electron microscopy

Electronmicroscopic studies with special reference to the adrenergic characteristics of the nerve terminals and the neuromuscular relationship in the isthmus have been performed (Paper II). The isthmic material used

for this purpose has been carefully dissected and muscular pieces of about one mm³ have been fixed in a glutaraldehyde-formaldehyde solution and postfixed in osmium tetroxide. Ultrathin sections were examined and photographed in a Philips EM 300 electron microscope.

Immunohistochemical and immunochemical methods

The occurrence of Vasoactive Intestinal Polypeptide (VIP) has been elucidated by means of immunohistochemical methods using specimens from isthmus as well as from the ampullary region (Papers VI and VII). Some specimens were immediately after removal frozen in propane-propylene at the temperature of liquid nitrogen. After freeze-drying, the preparations were fixed in diethylpyrocarbonate vapor and embedded in paraffin. Other specimens were fixed in formaldehyde buffer solution, frozen and sectioned in a cryostat. The sections were then processed for immunofluorescence (Coons et al. 1955) or immunoperoxidase staining (Sternberger 1974) with a previously characterized anti-VIP serum (Fahrenkrug and Schaffalitzky de Muckadell 1977).

Tissue VIP was quantified by radioimmunoassay (Paper VII). Materials were taken from the human female genital tract and stored frozen until analysis. After homogenization in acidified ethanol and centrifugation, the supernatant was decanted and the residue homogenized in acidified ethanol. After another centrifugation the combined supernatant fractions were evaporated to dryness in vacuo, and antiserum was added to reconstituted samples. Labelled VIP was added after an incubation period of 48 hours. The free and bound VIP was separated by absorption to plasma-coated activated charcoal (Fahrenkrug and Schaffalitzky de Muckadell 1977).

Radioimmunoassay of sex steroids

The cyclic stage was estimated on the basis of the patient's menstrual cycle, together with histological examination of endometrial material when possible. In order to obtain more precise definition of the patient's cyclic stage, plasma estradiol and progesterone levels were determined in blood drawn within one hour before operation. It was found in a separate study that during the 45-90 min long operation there was a marked reduction in plasma estradiol-17 β as well as progesterone and they both continued to fall in the course of the first postoperative day. The fall was less marked,

or absent, when the preoperative levels of the steroid were low. For this reason, immediately preoperative sampling was considered most adequate. A postoperative fall of sex steroids in plasma has previously been reported, not only in hysterectomized patients in which the ovarian blood supply is inevitably affected, but also after operations not influencing ovarian blood flow (Stone et al. 1975, Soules et al. 1980). These changes in plasma steroid levels may be caused by an afferent nerve reflex (Kehlet 1978, Engqvist et al. 1980).

The content of estradiol as well as progesterone was determined in isthmus and ampulla of Fallopian tubes (Paper V). The peritoneal covering was dissected away from the tubal muscular wall. By means of a 2 mm outer diameter (o.d.) probe introduced from the ampullary end, the ampullary-isthmic junction was localized and the oviduct divided at that point. A few mm of the adjacent isthmus and ampullary portions, as well as the infundibular part of the tube, were discarded. The preparations were then stored frozen at -18°C until analysing. The tissues were thawed and then digested in a 0.5 ml mixture containing 5 % sodium dodecyl sulfate and 0.5 N NaOH (Batra and Bengtsson 1976). The digested material was extracted three times with 3 volumes of ethyl acetate. The combined extracts were evaporated to dryness. However, the dried extracts contained a significant amount of sodium dodecyl sulfate, which was removed before radioimmunoassay by Sephadex LH-20 chromatography.

Progesterone was measured by a radioimmunoassay method similar to that described by Youssefnejadian et al. (1972). The antiserum was found to be highly specific for progesterone. It was raised in sheep against bovine serum albumin conjugated with 11α -hydroxyprogesterone. Plasma was extracted in petroleum ether and progesterone determined when the solvent was evaporated. After addition of antiserum and ^3H -progesterone to samples or standards, they were mixed and equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal. The sensitivity of the assay was 25 pg/ml and the inter- and intra-assay coefficients of variation were 18.7 and 10.5 %. Cross-reaction of the antiserum was < 10 % to 17α -hydroxyprogesterone and < 0.05 % to 20α -hydroxyprogesterone.

The procedure for radioimmunoassay of estradiol was that described by Lindberg et al. (1974). The antiserum was raised in sheep against estradiol- 17β -oxime conjugated with bovine serum albumin. After extraction of plasma with ethyl acetate, antiserum and ^3H -estradiol were added. The mixture was

equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal. The assay sensitivity was 5 pg estradiol/ml and the inter- and intra-assay coefficients of variation were 16.2 and 9.9 %. Cross-reaction of the antiserum to estradiol-17 α was 0.7 %, and it was 11 % for estrone (Batra et al. 1979a and b).

On the basis of the levels of estradiol and progesterone in plasma the material was separated in three main stages, secured when possible with the patient's menstrual record and the histological examinations of endometrial material: the follicular phase included women with plasma progesterone $\leq$ 2 ng/ml and estradiol $\leq$ 100 pg/ml, the ovulatory phase consisted of women with the same plasma progesterone levels, but estradiol $>$ 100 pg/ml, and the luteal phase included those having progesterone $>$ 2 ng/ml and a range of estradiol values.

In vitro model system

The tubal material used for in vitro recordings was immediately after removal placed in ice-cold Krebs-Ringer buffer solution. The tubal connective tissue was removed and the ampullary-isthmic junction was identified by means of a probe (2 mm o.d.) introduced from the ampullary lumen. The oviduct was divided at the identified junction and pieces from isthmus and ampulla were oriented so that primarily circular or longitudinal smooth musculature was used for isometric registration of motor activity. The bath contained a modified Krebs-Ringer solution, and the temperature of the bath was thermostatically maintained at 37°C and aerated with O₂/CO₂ to give a pH of 7.4. When the preparations after a period of 45-60 min started to contract regularly, various drugs were added to the baths. The motor activity and the change produced were defined in terms of frequency, amplitude of the spontaneous contractions, or by the planimetrically determined area under the recorded curves during a limited period of time.

The size of the preparations used, with the entire thickness of the wall intact, does not seem to pose any problems of oxygenation or drug diffusion. In order to estimate the thickness of the various layers of the tubal wall (Paper III), avoiding as far as possible shrinkage artefacts, pieces from ampulla and isthmus were frozen to -80°C, sectioned in a cryo-state, stained and measured with a device fitted to a microscope eye-

piece. The isthmus circular muscle layer had a mean thickness of 0.43 mm and the outer longitudinal layer a mean thickness of 0.26 mm. Preparations taken for fixation at the end of the pharmacological in vitro experiments did not show any electronmicroscopic signs of hypoxia (Sporrong, personal communication). Moreover, the frequency of the phasic contractions of the smooth musculature (Paper III), which has a higher demand for oxygenation than tonically contracting smooth musculature and is therefore more sensitive to hypoxia (Hellstrand 1979) was in the same order of magnitude as the frequency recorded in microdissected smooth muscle preparations (Lindblom et al. 1980). In smooth musculature the energy stores of phosphocreatinine and adenosine triphosphate (ATP) are relatively small, e.g. in the bovine mesenteric artery these high energy compounds will be sufficient only for a few minutes' contraction (Lundholm and Möhme-Lundholm 1965). In view of this we found (Paper III) that the spontaneous contractility in the preparations gradually ceased and was abolished within 8-10 min after O_2 had been changed with N_2 in the gassing system of the organ bath. On the other hand, the preparations continued to contract normally (observation time 4 hours) in a glucose-free but oxygenated (95 % O_2 , 5 % CO_2) buffer solution.

In order to elucidate whether the pharmacological properties of the α - and β -adrenoceptors may be involved in any cyclic changes of neuromuscular functions of the Fallopian tube, a quantitative pharmacological characterization of these receptors was carried out (Paper IV). The spontaneous contractions of the preparations were abolished by means of K^+ depolarisation of the smooth muscle cell membranes, the NaCl in the original Krebs-Ringer solution being exchanged by KCl in equimolar proportions (Evans et al. 1958).

During studies of motor inhibition (Paper IV), the adrenergic α -receptors were irreversibly blocked with phenoxybenzamine hydrochloride which also inhibits extraneuronal uptake of the agonist. The preparations were rinsed after 30 min, and cocaine was added in order to inhibit the neuronal uptake of noradrenaline, which was given in cumulative doses. Adrenergic β -receptors were blocked by the competitive β -antagonist, propranolol.

When testing the contractile response (Paper IV), the β -receptor was blocked with propranolol. Phenylephrine was used as agonist and the α -receptor antagonist, phentolamine, was added in varying concentrations. Intra- and extra-

neuronal uptake of the amine was inhibited with cocaine and normetanephrine, respectively. Semilogarithmic dose-response curves for the agonist were constructed. The concentration of the agonist giving half maximal response (ED_{50}) was compared before and 15 min after administration of the corresponding antagonist in increasing doses. On the basis of ED_{50} values, the dissociation constant (K_B) for the receptor antagonist complex was calculated (Furchgott 1972) and compared regarding cyclic phase. The competitive nature of the antagonism was secured by the Arunlakshana and Schild (1959) plot.

Electrical field stimulation (Papers II and VIII) was applied by two platinum electrodes mounted in the above-mentioned baths in order to activate the nerves in the preparations. The selective stimulation of the nerves was secured by adding tetrodotoxin in control experiments.

Computer analysis

Part of the material included in the in vitro studies has been computer analysed (Paper III). This was done in order to obtain better information about the relationship between the noradrenaline-induced motor activity and the sex hormone stage as defined by estradiol and progesterone in plasma. The initial data on tubal motor activity were registered as a number of points irregularly spaced over a two-dimensional map, which was divided into squares (grids). Each grid was then assigned a value equal to the average of the observations inside the square. Depending on the chosen size of the grids and the primarily irregular mapped values, some squares did not include any observations. The values of these squares were approximated from the closest observations of adjacent squares. In areas with few observations the values were then smoothed by a second order interpolation (Bladh and Jern 1980). The results were depicted by a 3-color ink jet plotter (Smeds 1973).

RESULTS AND COMMENTS

Regional adrenergic innervation

The development of the Falck-Hillarp fluorescence histochemical method has made it possible to demonstrate the adrenergic innervation with high precision and specificity. Thus, the human Fallopian tube was found to receive a rich supply of fluorescent nerves (Brundin and Wirsén 1964, Owman et al. 1967), in agreement with the presence of fluorometrically measurable noradrenaline in the organ (Owman et al. 1967). The innervation was found to be more dense in the isthmus than in the ampullary region, where the nerves were primarily located in the smooth muscle layers (Owman et al. 1967, Kubo et al. 1970). The concentration of noradrenaline was higher in the isthmus compared to the ampullary region (Owman et al. 1967). In a study by Dujovne et al. (1976) such a relationship was found only in the proliferative phase.

In order to evaluate any regional differences in the adrenergic innervation density along the Fallopian tube, high performance liquid chromatography has been adapted for noradrenaline quantification (Hansson and Rosengren 1979). The method allows the analysis of small specimens. The noradrenaline concentration was confirmed to be significantly higher in the isthmus than in the ampulla and fimbriated end, not only in the proliferative phase (Paper I).

Determinations of noradrenaline carried out in consecutive parts throughout the length of the tube showed certain regional differences (Paper I). The concentration of noradrenaline fell progressively in direction from the uterus towards the ampulla. A more abrupt decline was found when the ampullary-isthmus junction was passed. In the rest of the ampulla and in the fimbriated end a relatively low noradrenaline concentration was maintained. The highest concentration of noradrenaline in the isthmus, where the adrenergic nerves are primarily related to non-vascular smooth musculature (Owman et al. 1967, Kubo et al. 1970), is consistent with the idea that the musculature in this region may act as a sympathetically innervated sphincter mechanism. The regional measurements of the transmitter did not provide any clear evidence for a particularly prominent sympathetic influence in any part within the isthmus, which may therefore operate as a uniform structure in its motor, including sphincter-like, function.

Neuromuscular relationship

Adrenergic nerves characterized by their 50-60 nm large synaptic vesicles, and located both within and outside the smooth muscle cell bundles, have been distinguished in the human Fallopian tube by electron microscopy (Ishii 1972, Paper II). This is in contrast to observations by Daniel et al. (1975), who found adrenergic nerves primarily in the peripheral region of the smooth musculature, which contains chiefly collagen and blood vessels. In the same study it was concluded that there is no anatomical basis for peristalsis or for any other complex neurogenic control system in the human Fallopian tube. Ishii (1972) found only rarely direct synaptic contacts between nerves and smooth muscle cells. The adrenergic neuromuscular distance was 60-100 nm, and only few close contacts with a distance of 20 nm were demonstrated, from which it was concluded that the sympathetic nerve supply in the human Fallopian tube does play an important role in the control of ovum transport by peristaltic motion. Neuromuscular distances as short as 60-100 nm have recently been confirmed (Paper II). According to Bennett (1972) and Burnstock (1970), transmitter release is effective when the neuromuscular distance is up to 300-1000 nm. Electric field stimulation of tubal preparations under circumstances when only the nerves are activated has made it possible to demonstrate that the tubal adrenergic nerves can release sufficient amount of noradrenaline to produce a frequency-dependent motor response (Paper II).

Thus the isthmus seems to be better equipped with adrenergic neuromuscular contacts than previously thought. There are also reasons to believe that the neuromuscular distance is small enough for effective transmitter diffusion and, hence, that the adrenergic innervation of the human Fallopian tube is of functional importance.

Hormonal influence on spontaneous motor activity

The relationship between sex steroid levels and tubal motor activity was studied in terms of the frequency of the spontaneous contractions. This frequency showed similar patterns, both regarding cyclicity and regional distribution, as those reported by Lindblom et al. (1980) for microdissected smooth muscle preparations. Thus around ovulation, the frequency was higher in the circularly than in the longitudinally oriented musculature. Computer analysis of the material and subsequent mapping by means

of a three-dimensional color ink plotter showed that there is a steadily increasing frequency during follicular phase towards a maximum around ovulation, which is followed by a return to lower frequencies during the luteal phase.

Also patients taking contraceptive pills, pregnant, or in menopause were studied (Paper III). The spontaneous phasic activity in tubes obtained from women taking combined steroid contraceptives agreed with what could be expected on the basis of the relation found between plasma steroid levels and frequency in patients with normal menstrual cycles. The mean frequency was, however, higher than that reported by Lindblom et al. (1980). The muscle preparations from menopausal women usually contracted with a slightly lower frequency than that found in women receiving steroid contraceptives, which in turn well corresponded to the higher estradiol values in the latter group of patients. As could be expected, the frequency of the spontaneous contractions during pregnancy, with its high plasma steroid levels, agreed with the frequency found during the luteal phase.

Cyclic fluctuations in noradrenaline-induced motor activity

In order to illustrate the net response that might be expected following sympathetic neurotransmitter release in the Fallopian tube, the effect of noradrenaline on tubal motor activity was studied. The noradrenaline-induced changes in motor activity in the isthmic and ampullary parts of the Fallopian tube were analysed in vitro (Paper III). A standard dose of noradrenaline ($3 \times 10^{-6} \text{M}$) was chosen which produces an α -receptor-mediated effect between the half maximum and maximum response. This concentration in the region of the receptor is in the same order of magnitude as the estimated instantaneous concentration of noradrenaline occurring in the synaptic cleft of a neuromuscular contact during the passage of a sympathetic nerve impulse (Bennett 1972).

The parameters for motor activity found to be of particular interest were the frequency of the phasic contractions and the area under the recorded curve. When the adrenergic neurotransmitter was thus added to the organ bath, the frequency of the contractions in the circular smooth musculature

of both isthmus and ampulla was lowered during the follicular and luteal phases, whereas little or no change occurred during the ovulatory period.

In order to obtain a total picture of the composite changes in contractile activity of the Fallopian tube, the surface area under the recorded curve was determined during a 3-min period just before and after noradrenaline administration to the bath. The base line was defined as the lowest level of tone from which the individual deflections originated during the total 6-min measurement period. A marked increase of contractile activity was found around ovulation in all types of tubal preparations. Also in the follicular phase there was an increase, though less pronounced, in contractile activity of the longitudinally oriented musculature. The circular musculature responded with reduced activity during this phase. In the luteal phase all preparations, except the isthmic longitudinal, responded to noradrenaline with a clear reduction. During menopause, treatment with steroid contraceptives, or in pregnancy noradrenaline always reduced motor activity.

The results confirm not only that there is a relationship between the hormonal state and the spontaneous motor activity in the tube, but also that there are cyclic fluctuations in the way the tube responds to the adrenergic transmitter. This response is not merely expressed as a quantitative alteration in the motor activity, but also as an alteration in the direction of the noradrenaline-induced response. The effect may be mediated through an altered balance between tubal α - and β -adrenergic receptors (cf. Seitchek et al. 1968), as suggested for the human oviduct by Moawad et al. (1976) and Owman et al. (1976), and further substantiated in the detailed analysis described in Paper IV.

Cyclic effects on adrenergic nerve function

Adrenergic nerves in the Fallopian tube belong to the special system of "short adrenergic neurons" (Owman et al. 1966), which have a particular sensitivity to the effect of sex steroids (Owman and Sjöberg 1977). There is evidence for such influences also on the adrenergic nerves of the Fallopian tube, though the results have not been unequivocal. Some authors (Owman et al. 1967, Dujovne et al. 1976) demonstrated with fluorometric methods a higher noradrenaline level in the secretory compared to the follicular phase, in contrast to Moawad et al. (1977), who found that the concentration is higher in the follicular and lower during the luteal period, with the lowest values around the time of ovulation. Also the data

from fluorescence histochemical techniques are conflicting. Owman and Sjöberg (1976a) showed on rabbits that there is a marked increase in the level of noradrenaline after administration of estrogen, and this was followed by a reduction to control levels when progesterone was added. The rate of turnover of catecholamines - an index of the functional state of sympathetic neurons in vivo - was higher in estrogen-treated rabbits (Takeda and Doteuchi 1976). Kennedy and Marshall (1977), giving 10 times lower estrogen doses, did not reveal any difference between rabbits treated with estrogen and with estrogen/progesterone. Thus, the level of plasma hormones, or rather the amount of steroid receptor available, may be of primary importance. Human oviducts have a larger number of fluorescent adrenergic terminals in the secretory phase compared to the follicular phase (Owman et al. 1967). On the other hand, Moawad et al. (1977) failed to demonstrate differences in adrenergic nerve fluorescence related to the hormonal phase.

The high performance liquid chromatography method was used to evaluate cyclic variations in tubal noradrenaline concentrations (Paper I). The noradrenaline concentration was higher during the luteal compared to the follicular phase, confirming the previous fluorescence histochemical and fluorometric observations in monkeys and humans (Owman et al. 1967, Dujovne et al. 1976). The consecutive preparations throughout the tube suggested a pattern of noradrenaline fluctuations, so that in the isthmus and in the fimbriated end there was a tendency for the values to be higher in the ovulatory compared to the luteal phase. This may reflect a specialized hormonal dependence in the function of the adrenergic innervation in the isthmus (i.e. sphincter-like) part of the human Fallopian tube.

The cyclic fluctuations in tubal noradrenaline are probably the net result of several biochemical processes in the adrenergic neurons, as investigated in some more detail for the uterine adrenergic innervation (cf. Thorbert 1978). Against the background of available information from the tubal nerves (e.g. Takeda and Doteuchi 1976), it can be assumed that the higher levels of noradrenaline presently found reflect increased sympathetic nerve activity in the tube.

Characteristics of adrenoceptors - relation to cyclic stage

The sympathetic neurotransmitter has a decreased potency on the circular

smooth musculature of the progesterone-dominated rabbit oviduct (Higgs and Moawad 1974, Rheume and Paton 1977). The effect of progesterone has been attributed to a decrease in α -receptor activity. This could not be verified by Rheume and Paton (1978), who found that progesterone treatment increases the maximal inhibitory effect of isoprenaline on the β -receptor but does not alter the sensitivity to the agonist, suggesting a change in number of β -adrenoceptors rather than in receptor affinity. This is in accordance with the results of Martin et al. (1970), who observed enhanced β -receptor activity in the oviduct of progesterone-treated rabbits.

In order to elucidate the pharmacological properties and the reactivity of the α - and β -adrenoceptors in human Fallopian tubes, a quantitative pharmacological characterization of the receptors was performed in Paper IV. The quantification of the adrenergic receptors with the use of full dose-response curves was more conveniently performed when the phasic contractions of the tubal preparations were abolished by means of potassium depolarization of the smooth muscle membrane (Evans et al. 1958). The relaxing effect of noradrenaline on the tubal smooth musculature was studied under conditions when the contractile response was inhibited by phenoxybenzamine. The log-dose response curve in the presence of increasing doses of propranolol showed a parallel shift towards higher agonist concentrations, confirming that the relaxation was mediated by β -adrenergic receptors. Based on this parallel shift the dissociation constant between the β -receptor and propranolol could be calculated for different cyclic stages and in different regions of the Fallopian tube. The results showed no statistically significant regional or cyclic variations in the affinity of propranolol for the tubal smooth muscle β -receptor.

The sympathomimetic contraction was studied under similar standardized conditions as the relaxation, using phenylephrine as agonist. A number of preparations, especially those from isthmus, did not respond to phenylephrine, probably due to the large concentration of β -receptors in the prominent isthmic circular muscle layer (Lindblom et al. 1979). In other preparations phentolamine lowered the sensitivity of the smooth musculature to phenylephrine without changing the slope of the log dose-response curve. The affinity of phentolamine for the α -receptor showed a significant difference in the circular smooth musculature when related to low versus moderate to high plasma levels of estrogen and progesterone in plasma: it was higher during the follicular phase compared to the remainder of the menstrual cycle.

No such difference was found for the longitudinal smooth musculature of the isthmus, whereas in the ampulla the affinity was lower in the follicular phase.

The dissociation constant for the α -receptor - antagonist complex (K_B) seemed to fall into two groups, with approximate values of 6×10^{-8} and 2×10^{-7} . It is well known that α -receptors (designated α_2 -receptors) are also demonstrable prejunctionally (Starke et al. 1977, Langer 1980). However, there are recent evidence suggesting the presence of both types of α -receptors postjunctionally (Lefkowitz and Hoffman 1980). The affinity of phentolamine for the postjunctional α_1 -receptor has been found to be higher than for the prejunctional receptor (Starke et al. 1975). The K_B values calculated for these pre- and postjunctional α -receptors seem to correspond well with the two populations of values found in the smooth musculature of the human Fallopian tube. It is possible that the two groups of K_B values represent two subtypes of α -receptors, each predominating during different parts of the menstrual cycle.

Relationship between levels of sex steroids in plasma and tubal tissue

Using the levels of plasma estradiol and progesterone as a tool to define cyclic stage it has been possible to demonstrate cyclic changes in (a) tubal adrenergic nerve function (Paper I), (b) the response of the tubal smooth musculature to the sympathetic neurotransmitter (Paper III), and (c) the adrenoceptors mediating the sympathetic neuromuscular response (Paper IV). It has been a general trend while studying the effects of estradiol and progesterone on various female reproductive tissues to relate the intensity of the effect to the concentration of hormones in the blood. It is, however, more reasonable to think that the biological activity will perhaps be governed by concentrations in the tissue. Determinations of the sex steroid concentrations in the endometrium and myometrium indicate that there is a poor correlation between these hormones in tissue and plasma (Batra et al. 1977, Batra and Bengtsson 1978, Runnebaum et al. 1978). No data were available on the human Fallopian tube.

The tubal levels of estradiol and progesterone were determined and related to protein content since the protein concentration varied significantly between the isthmus and ampulla, though there was no difference in regard to hormone cyclic phase (Paper V). The steroid concentrations did not differ

between the tubal regions. Mean values of estradiol were highest around the time of ovulation, while progesterone rose significantly during the luteal phase. The pattern of tissue concentration of the steroids resembled that of the plasma steroids, but there was a generally poor correlation between the individual plasma and tissue values, as shown in linear regression analysis.

In order to get an idea of the amount of estrogen and progesterone receptors available and the steroid accumulating capacity of the tissue, a tissue/plasma ratio of these steroids was constructed. Both isthmus and ampulla showed highest ratios for estradiol in the follicular phase, and for progesterone the highest values were found during the ovulatory period. In all phases and preparations, the concentration was considerably higher in tissue than in plasma which suggests that the Fallopian tube can be considered a target organ for female sex steroids. Recently, the existence of both estrogen and progesterone receptors in the human Fallopian tube has been demonstrated (Robertson et al. 1975, Muechler et al. 1976, Verhage et al. 1980). The progesterone receptor level in the entire Fallopian tube seems to be highest in what is designated as "late follicular phase" by Verhage et al. (1980), well corresponding to the highest progesterone ratio found during the time of ovulation in Paper V. According to Robertson et al. (1975), the concentration of estradiol receptors is highest during the follicular phase, both in isthmus and ampulla. Also this corresponds well with the highest tissue/plasma ratio of estradiol found during the follicular phase (Paper V).

Thus, the consistently high tubal motor activity, spontaneously and following sympathomimetic stimulation, found in vitro (Paper III) corresponds with high progesterone receptor concentration in the tissue. Following ovulation, when the plasma progesterone levels rise, there is a drop in the specific tissue binding of this steroid, which was associated with a reduced motor activity in the tube following noradrenaline administration. The effect of noradrenaline was less consistent, particularly in the longitudinal smooth musculature, during the follicular phase. This may reflect a progressive increment in α -adrenoceptor activity in relation to the β -receptors during the follicular phase (Moawad et al. 1976). In this phase there is a high concentration of both estrogen and progesterone receptors (Robertson et al. 1975, Verhage et al. 1980, Paper V).

Non-adrenergic, non-cholinergic motor effects

Two kinds of observations made in the course of the present studies have strongly indicated the existence of a non-conventional type of nerve-mediated motor inhibition in the human Fallopian tube: one is the presence of nerves containing VIP (Paper VI), which was found to markedly reduce the contractile activity (Wallés et al. 1980), another is the motor inhibition occurring during transmural nerve stimulation in spite of adrenergic and cholinergic blockade (Paper VIII).

Electrical field stimulation of tubal smooth muscle preparations with short pulse duration evokes a motor response that is either an increase or a decrease in motor activity, or often a biphasic response. Initially, the stimulation gives rise to a twitch contraction shown to be the result of an α -adrenoceptor activation (Paper II). Also when the adrenergic and cholinergic receptors are blocked, a reduced motor activity is still possible to evoke (Paper VIII). Addition of the nerve blocking agent, tetrodotoxin (Moore and Narahashi 1967), abolished the α -adrenergic response and also the reduced motor activity, showing that the response was mediated by stimulation of nerves in the preparations rather than an effect of direct smooth muscle activation. This is in agreement with the observation by Murcott and Carpenter (1977) who found evidence in the rat oviduct for a non-adrenergic inhibitory mechanism. Lindblom et al. (1979) have confirmed the presence of a non-cholinergic, non-adrenergic mechanism in the human oviduct.

Vasoactive intestinal polypeptide: innervation and motor action

Several peptides with possible neurotransmitter function have recently been shown to be widely distributed in peripheral organs. One of the most studied is VIP, a 28-amino acid peptide chain, which was originally isolated from porcine small intestine in 1970 by Said and Mutt. The sequence of the amino acids was also established by Mutt and Said (1974) and the substance was then synthesized by Bodanszky et al. (1974). Evidence is accumulating that the peptide is a neurotransmitter (cf. Fahrenkrug 1979), though final proof for this is still lacking, mainly because an antagonist for this peptide is not yet available.

By means of immunochemistry, VIP has been demonstrated to occur in the female genital tract (Alm et al. 1977, Larsson et al. 1977). In human tissues VIP-immunoreactivity has been found in the Fallopian tube especially in the smooth muscle layers but also beneath the epithelium of the isthmus part. In the ampullary region and in the fimbriated end, only scattered VIP terminals were observed (Paper VI). VIP has been possible to localize in nerve cell bodies, already at 18-23 weeks of pregnancy, occurring in clusters in the paracervical tissue, which suggests that utero-vaginal VIP nerves may have a local origin, analogous to the short adrenergic neurons. This is supported by denervation experiments in the cat which have shown that VIP nerves originate from paracervical ganglia (Alm et al. 1980). The content of VIP measured by radioimmunoassay was found to be significantly higher in the isthmus of the Fallopian tube than in the rest of the tubal tissue (Paper VII), showing a good correlation between the immunohistochemical and immunochemical findings.

VIP is known to have a number of biological effects, among others it relaxes non-vascular smooth muscles (Piper et al. 1970, Vagne et al. 1976, Ottesen et al. 1981). Exposure of smooth muscle preparations from the human female reproductive tract to VIP induced a dose-dependent relaxation of the tubular isthmus smooth musculature, both in circularly and longitudinally oriented preparations (Wallis et al. 1980, Paper VII). Also the uterine cervical region, which like the tubal isthmus has a dense VIP innervation, showed a dose-dependent reduction in motor activity in the presence of exogenous VIP. On the other hand, the poorly VIP-innervated corpus uteri did not react even to high doses of VIP. Thus, the response of the tissue to exogenous VIP is well correlated to the local density of VIP-immunoreactive nerves, suggesting that VIP plays a role in the control of tubal smooth muscle activity.

Evidence for VIP in the non-adrenergic, non-cholinergic motor effects

Administration of VIP in vitro induced a reduction in tubal motor activity that was sometimes immediate, but in other instances the effect reached its maximum more slowly (Paper VIII). The discrepancy between the immediate response occurring during nerve stimulation and the sometimes more progressive change in motor activity following exogenous VIP administration might be due to the relatively longer diffusion distance for the exogenous VIP, and does not contradict that the nerve-induced reduction

in motor activity is the result of local VIP release. For lack of a specific VIP-receptor blocking agent, another approach was used to show that the non-adrenergic, non-cholinergic motor inhibition of the tube was mediated by a VIP-receptor. Thus electrical field stimulation of the tubal nerves (in the presence of adrenergic and cholinergic antagonism) was carried out with a high concentration of VIP in the organ bath. The intention was to occupy the VIP receptors, and thereby block the effect of any nerve-released VIP on the smooth musculature. The decrease in tension obtained after addition of exogenous VIP was balanced by vasopressin in order to maintain the control level of tension before the electric field stimulation was started. Under these circumstances nervous activation failed to reduce the smooth muscle activity. It should be noted that vasopressin has not been found in tubal nerves, and it does not in itself influence the nerve-induced non-adrenergic, non-cholinergic reduction in motor activity. The VIP-inhibitory effect did not seem to be non-specific e.g. involving desensitization (Triggle 1980), since the preparation still responded with a pronounced reduction in motor activity after addition of the β -receptor agonist, terbutaline (under these conditions, the adrenergic mechanisms were blocked by bretylium which does not affect the adrenoceptors). The experiments favor our view that VIP is responsible for the neurogenic non-adrenergic, non-cholinergic inhibition of motor activity in the isthmus of the human Fallopian tube.

GENERAL SUMMARY OF OWN INVESTIGATIONS

1. The studies have been performed on human Fallopian tubes obtained during routine gynecological operations from more than 500 women.
2. The material was grouped in the three cyclic stages - follicular, ovulatory and luteal - primarily on the basis of plasma concentrations of estradiol - 17β and progesterone determined by radioimmunoassay.
3. Tubal noradrenaline, exclusively present in adrenergic nerves, was determined by high performance liquid chromatography. The highest concentration was found in the isthmus, without any clear local variation. Detailed regional analysis indicated a maximum concentration in the isthmus and the fimbriated end during ovulation, whereas the ampulla had its highest noradrenaline level during the luteal phase.
4. The adrenergic nerve terminals could be identified by electron microscopy and were found to have a close association with the smooth muscle cells, the neuromuscular distance being as short as 60-100 nm.
5. The functional nature of the adrenergic innervation was confirmed by transmural electrical stimulation of the nerves, which were able to release a sufficient amount of sympathetic transmitter to produce a frequency-dependent motor response, in these experiments defined as an α -receptor mediated twitch contraction.
6. Cyclic effects on tubal motor activity were studied in four types of smooth muscle preparations - circular and longitudinal musculature of isthmus and ampulla - in terms of spontaneous motor activity and noradrenaline-induced alterations in the activity. The frequency of the spontaneous contractions increased progressively during the follicular phase to become maximal around ovulation. Noradrenaline in general potentiated the difference in frequency between the ovulatory phase on the one hand and the follicular and luteal phases on the other. The transmitter markedly increased the area under the recorded curve during the ovulatory phase in all types of smooth muscle preparations.

7. The α - and β -adrenergic smooth muscle receptors were subjected to quantitative pharmacological characterization in the four types of smooth muscle preparations. The characteristics of the β -receptor did not vary during the menstrual cycle. The properties of the α -receptor resembled that of the α_1 -subtype when plasma estradiol -17 β and progesterone were both minimum (follicular phase) and the α_2 subtype when these steroid levels were moderate to high (the remainder of the menstrual cycle).
8. The tissue concentrations of estradiol and progesterone were measured radioimmunologically in the isthmus and ampulla during the follicular, ovulatory and luteal phases. There was a poor individual correlation between plasma and tissue values. The tissue/plasma ratio of the steroid concentrations was calculated to estimate the steroid accumulating ability of the tissue. This was highest for estradiol during the follicular phase and during ovulation for progesterone.
9. The distribution of vasoactive intestinal polypeptide (VIP) has been mapped out by a combination of immunohistochemical and immunochemical techniques. The highest concentration of VIP nerves was found in the (non-vascular) smooth musculature of the tubal isthmus and uterine cervix. The nerves probably originate locally, from ganglia in the paracervical region.
10. VIP induced a marked dose-dependent reduction in tubal motor activity, and pharmacological evidence is provided to show that this is mediated by a non-adrenergic, non-cholinergic neurogenic mechanism.

ACKNOWLEDGEMENTS

The accomplishment of this work was made possible by kind help of many persons..I wish to express my sincere gratitude to;

Professor Christer Owman for his invaluable scientific guidance and enthusiastic support throughout the work.

Associate Professor Nils-Otto Sjöberg, my other tutor, for his valuable and fervent continuous support.

I am grateful to Associate Professors Bengt Walles, Satish Batra and Frank Sundler for introduction in methodological details and for fruitful discussions.

Professors Lars-Philip Bengtsson, Birger Åstedt, Bengt Falck and Evald Rosengren for the use of facilities at the Department of Obstetrics and Gynecology, Department of Histology, and Department of Pharmacology, University of Lund.

Mrs Elisabeth Albertsson, Eva Engelbert, Barbro Gustavsson, Bodil Nilsson, Joanna Nilsson, Astrid Persson, Kristina Källstrand and Mary-Ann Sällström for skilful technical assistance.

Mrs Gun Almarker, Eva Kroon and Ingrid Stedman for perfect secretarial work and to Agneta Persson for excellent help with the illustrations. My colleagues and the staff, particularly in the surgical unit, at the Department of Obstetrics and Gynecology and at the Department of Histology for never failing loyalty.

The studies were supported by grants from the Faculty of Medicine, University of Lund, the Swedish Medical Research Council (grant No 14X-5680), the Ford Foundation (grant No 680-0383A), Förenade Liv Mutual Group Finance Company, Maggie Stephens Foundation, Royal Physiographic Society in Lund and Anders Otto Swärd Foundation.

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Regional and Cyclic Variations in Catecholamine Concentration of the
Human Fallopian Tube

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Running title:

Catecholamine levels in human Fallopian tube

ABSTRACT

Catecholamines were measured in various regions of the human Fallopian tube at different cyclic stages with high performance liquid chromatography; these stages were defined from plasma estradiol and progesterone levels. The tube contained minute amounts of epinephrine, low concentrations of dopamine, and approximately 250-800 ng/g of norepinephrine. Measurements of norepinephrine in consecutive sections along the organ showed clear regional variations. The highest concentration was found in the isthmus. There was a consistently higher norepinephrine concentration during the luteal compared to the follicular phase. The highest norepinephrine values in the isthmus and in the fimbriated end were found at the time of ovulation.

Fluorescence histochemical studies of the human Fallopian tube have demonstrated a rich supply of adrenergic nerves, in agreement with the presence of fluorometrically measurable norepinephrine in the organ (Owman, Rosengren and Sjöberg 1967; Kubo, Kawano and Ishii 1970; Dujovne, deLaborde, Carril, Cheviakoff, Pedroza and Rosner 1976; Moawad, Kim, Zuspan, Chagrasulis, Pishotta and Zuspan 1977). The innervation of the non-vascular smooth muscle is considerably more dense in the isthmus than in the ampullary region. In the rabbit oviduct the tissue level and turn-over rate of norepinephrine have been found to be associated with changes in sex steroid levels (Bodhke and Harper 1973; Takeda and Doteuchi 1976; Owman and Sjöberg 1976). The junctional region between the ampulla and isthmus functions in this animal as a sympathetically innervated smooth muscle sphincter (Brundin 1965), which seems to be important in ovum fertilization and transport. In the human tube, the ovum is retained in the ampullary region by a "tube locking" mechanism during fertilization (Pauerstein and Eddy 1979). Since the sex steroids and the adrenergic innervation represent major factors governing tubal motility (for references, see Harper, Pauerstein, Adams, Coutinho, Croxatto and Paton 1976), studies were carried out on human Fallopian tubes to elucidate (a) regional differences in the concentration of the sympathetic transmitter along the organ to indicate regional difference in the innervation density, and (b) cyclic variations in tissue concentrations of the amine as an index of hormonal influences on sympathetic nerve function.

MATERIALS AND METHODS

Material

Human tubal tissue was obtained by abdominal hysterectomy (because of adenomyosis, myoma, or preinvasive carcinoma of the cervix), salpingo-oophorectomy (for benign ovarian cyst), or salpingectomy (for sterilization). One or both Fallopian tubes were taken from 48 adult, regularly menstruating

patients aged 32 - 52 years. No specimens taken from patients receiving any hormone treatment were included.

Morphine chloride (10 mg) and scopolamine bromide (0.4 mg) were given as preanesthetic medication. Anesthesia was induced by pentothal sodium administered i v together with pethidine chloride, followed by inhalation of a mixture of dinitrous oxide and oxygen. Succinylcholine iodide was given initially i v as muscle relaxant, followed by pancuronium bromide.

Definition of hormonal state

The cyclic stage was estimated on the basis of the patient's menstrual cycle as noted in the record, usually together with histological examination of endometrial material obtained by curettage of the uterine cavity during the operation, or sectioned and stained preparations from the removed uterus.

Blood (approximately 10 ml) was drawn by venopuncture for determination of progesterone and estradiol levels in plasma. The sample was taken during a standardized time interval - within 1 hr before opening of the abdomen - since it has previously been observed (Helm, Owman and Sjöberg 1981) that the plasma sex steroid levels are changed markedly during the course of the operation.

Progesterone was measured by a radioimmunoassay similar to that described by Youssefnejadian, Florensa, Collins and Sommerville (1972). The antiserum was raised in sheep against bovine serum albumin conjugated with 11α -hydroxy-progesterone. Usually, 25 to 100 μ l of plasma were extracted twice with petroleum ether, and progesterone was determined after evaporating the solvent. After addition of antiserum and of ^3H -progesterone to samples or standards, they were mixed and equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal. The sensitivity of the assay was 25 pg/ml, and the inter- and intraassay coefficients of variation were 18.7 and 10.5 %, respectively. Cross reaction of the antiserum

was less than 10 % to 17 α -hydroxyprogesterone and less than 0.05 % to 20 α -hydroxyprogesterone.

The procedure for radioimmunoassay of estradiol was that described by Lindberg, Lindberg, Martinsson and Johansson (1974). The antiserum was raised in sheep against estradiol-17- β -oxime conjugated with bovine serum albumin and used in a dilution of 1:100,000. After extraction of 1 ml of plasma with diethyl ether, antiserum and ^3H -estradiol were added. The mixture was equilibrated at +4 $^{\circ}\text{C}$ overnight. The unbound steroid was removed by addition of dextran-coated charcoal. The assay sensitivity was 5 pg estradiol per ml, and the inter- and intraassay coefficients of variation were 16.2 and 9.9 %, respectively. Cross reaction of the antiserum to estradiol -17 α was 0.7 % and it was 11 % for estrone (Batra, Bengtsson & Sjöberg, 1979; Batra, Sjöberg & Thorbert, 1979).

On the basis of the (preoperative) plasma levels and confirmed by the patient's menstrual record and the histological examinations, the material has been separated into the following main stages: The follicular phase included women with plasma progesterone ≤ 2 ng/ml and estradiol ≤ 100 pg/ml, the ovulatory phase women had the same plasma progesterone levels, but estradiol levels were > 100 pg/ml, and the luteal phase group had progesterone levels > 2 ng/ml.

Tubal preparations

Immediately after removal of the tubes, the surrounding connective tissue was dissected away from the muscle wall. A thin (2 mm outside diameter) probe was introduced into the lumen from the ampullary end in order to localize the ampullary-isthmus junction, where the oviduct was divided. The ampulla and isthmus were used separately for catecholamine determinations. In part of the material (see Table 1) the ampulla and isthmus were each divided into 5 consecutive portions of approximately equal size, and the fimbriated end

of the tube was taken for separate analysis. All preparations were gently blotted on filter paper, weighed, and placed in 5 ml of 0.4 M perchloric acid.

Determination of catecholamines

The ampullary and isthmic preparations, weighing approximately 900 and 170 mg, respectively, were homogenized and centrifuged for 5 min at 10,000 x g. To 10 ml of the supernatant, 2 ml of 10 % EDTA, 1 mg ascorbic acid, and 30 ng DL-isoproterenol hydrochloride (Sigma) were added as internal standard. The catecholamines were then adsorbed onto activated aluminum oxide. The sample was added to 0.2 g alumina and the pH of the mixture was quickly adjusted to 8.6 with a sodium hydroxide solution. The alumina was washed four times with 10 ml doubly distilled water. The catechols were removed from the alumina with 1 ml of 1M perchloric acid.

The catecholamines in the eluates were then determined by high pressure liquid chromatography with electrochemical detection using a slight modification of the method described by Hansson, Edholm, Agrup, Rorsman, Rosengren and Rosengren (1978). A Waters model 6000 A pump was used together with a Rheodyne Model 7121 sampling valve injector with a 100 μ l loop. Assay was carried out by a thin-layer amperometric detector (Bioanalytical Systems Inc., Model L-10). The column (4.6 x 250 mm) was packed with 5 μ ion-exchange material (Nucleosil 5SA), and the column and detector were operated at 45°C. The elution was performed by a solution containing 3.2 g sodium hydroxide, 5.5 g sodium acetate, 7.7 g citric acid, and 1.4 ml glacial acetic acid per l. The flow rate was 1.4 ml/min and the working electrode potential was set at +0.75 V versus Ag/AgCl as reference electrode.

The precision for repetitive determination of the three catecholamines determined was evaluated with the internal standard, and was found to be 3%

for norepinephrine, 4.6 % for epinephrine, and 4.6 % for dopamine. The plot of peak height versus the amount of injected amines was found to be linear over the concentration range of interest. The detection limit for the catecholamines in the samples was 250 pg.

The results obtained with this high pressure liquid chromatography method were on several occasions compared to those found when a reversed-phase liquid chromatographic system was used. In these cases a column containing Nucleosil C₁₈ was used instead of the cation-exchange resin. The correlation between the two methods was high. The specificity of the methods was further examined by addition of small amounts of authentic material to the sample. No inhomogeneities in the peaks examined could be observed.

RESULTS

Table 1 shows the mean values for the plasma concentrations of estradiol and progesterone in the three groups of patients created according to the principles given above to define cyclic stage.

The human Fallopian tube contained minute amounts of epinephrine, a low concentration of dopamine, and substantial concentrations of norepinephrine (Table 2).

The concentration of epinephrine (Table 2) was higher in the isthmus than in the ampulla and fimbria; there was no significant difference in concentration between the latter two regions. There were no clear signs of cyclic fluctuations in the epinephrine levels.

The isthmus region also contained the highest concentration of dopamine (Table 2). There was a consistently higher concentration of this amine during the luteal compared to the follicular phase (the difference being statistically significant in the isthmus and ampulla). During ovulation the dopamine concentration was intermediary between the levels in the other two phases.

The norepinephrine concentration was found to be significantly higher in the isthmus than in the ampulla or fimbria (Table 2). The norepinephrine determination carried out on consecutive preparations throughout the length of the tube provided evidence for a regional variation (Fig. 1). In the direction from the uterus towards the ampulla there was a progressive slight fall in the norepinephrine concentration. A more prominent reduction was seen in the ampullary preparations taken nearest to the isthmus. In the rest of the ampulla and in the fimbriated end a constant, relatively low norepinephrine concentration was maintained.

There were clear cyclic variations in the norepinephrine concentration, illustrated both in Table 2 and in Fig. 1. The concentration of norepinephrine was consistently higher during the luteal compared to the follicular phase. In the isthmus preparations there was even a tendency for the norepinephrine values in the ovulatory phase to exceed those found in the luteal phase (Fig. 1), whereas the amine values in the ampulla during ovulation usually were the same as found in the luteal phase, or intermediary between those and the concentrations measured in the follicular phase (Fig. 1). Also the fimbriated end of the tube had highest norepinephrine values during ovulation (Table 2 and Fig. 1), though the difference compared with the follicular phase was only at the limit of statistical significance.

DISCUSSION

Fluorescence histochemical studies have shown that norepinephrine in the human tube is present in nerves. Most of the adrenergic nerve terminals in the isthmus are non-vascular and they are primarily located in the smooth muscle layers of the organ, whereas the adrenergic innervation in the ampullary and fimbriated regions to a major extent is related to blood vessels (Owman et al. 1967; Kubo et al. 1971; Moawad et al. 1977). The small amount of

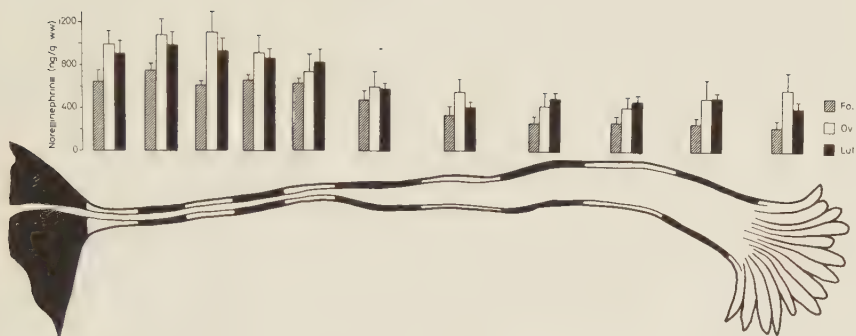


Fig. 1 Tissue concentrations of norepinephrine (ng/g wet weight) in consecutive parts throughout the length of the human Fallopian tube (5 preparations of equal length from the isthmus and ampullary regions, respectively) during the follicular (Fol; n = 5 for isthmus and ampulla, 14 for fimbria), ovulatory (Ov; n = 6 for isthmus and ampulla, 7 for fimbria) and luteal (Lut; n = 14 for isthmus and ampulla, 13 for fimbria) phases of the menstrual cycle.

TABLE 1

Radioimmunological determination of preoperative plasma concentrations of sex steroids in the three groups of patients created to define cyclic stage of the tubal material into follicular, ovulatory, and luteal phases. Values are means $\pm$ SEM, n = number of determinations.

Cyclic stage	n	Estradiol (pg/ml)	Progesterone (ng/ml)
Follicular	12	52.0 $\pm$ 6.2	0.48 $\pm$ 0.12
Ovulatory	12	249.7 $\pm$ 43.9	0.38 $\pm$ 0.09
Luteal	24	131.4 $\pm$ 12.1	7.73 $\pm$ 0.86

TABLE 2

Chemical determinations of tubal catecholamines (expressed as ng per g tissue wet weight) in the isthmus, ampulla, and fimbriated end during the follicular, ovulatory, and luteal phases of the menstrual cycle. Mean values $\pm$ SEM, number of determinations within parenthesis. The material includes that illustrated in Fig. 1; in the case of the fimbria it is identical with the material presented in Fig. 1.

Region	Dopamine (ng/g ww)			Norepinephrine (ng/g ww)			Epinephrine (ng/g ww)		
	Follicular	Ovulatory	Luteal	Follicular	Ovulatory	Luteal	Follicular	Ovulatory	Luteal
Isthmus	26.4 $\pm$ 3.3 (12) *** +	38.9 $\pm$ 9.1 (11) * +	49.5 $\pm$ 6.6 (23) * +	516.5 $\pm$ 37.9 (12) *** ++	724.5 $\pm$ 116.3 (11) * +	788.4 $\pm$ 75.1 (24) ***	13.6 $\pm$ 4.5 (12) **	16.7 $\pm$ 7.8 (9)	13.9 $\pm$ 5.1 (23) *
Ampulla	13.6 $\pm$ 1.7 (12) ++	18.2 $\pm$ 3.9 (12)	30.9 $\pm$ 4.2 (24)	286.2 $\pm$ 32.4 (12) +	400.2 $\pm$ 80.8 (12)	426.7 $\pm$ 39.6 (24)	0.6 $\pm$ 0.4 (12)	3.9 $\pm$ 1.3 (11) +	1.9 $\pm$ 0.6 (24)
Fimbria	16.6 $\pm$ 2.0 (4) **	23.1 $\pm$ 4.6 (7) * +	26.4 $\pm$ 4.9 (13) **	241.5 $\pm$ 60.4 (4) ***	579.4 $\pm$ 166.9 (7)	404.8 $\pm$ 63.4 (13) ***	2.7 $\pm$ 1.4 (3)	3.9 $\pm$ 1.3 (5)	1.9 $\pm$ 0.4 (10) *

Comparison between mean values evaluated with Student's t-test. Regional variations: *** $p < 0.001$; ** $0.001 < p < 0.01$; * $0.01 < p < 0.05$ for isthmus vs ampulla, ampulla vs fimbria, fimbria vs isthmus (the first region mentioned in the comparisons bears the symbol).

Cyclic variations: ++ $0.001 < p < 0.01$, + $0.01 < p < 0.05$ for follicular vs luteal, ovulatory vs follicular and luteal vs ovulatory phases (the first phase mentioned in the comparisons bears the symbol). Non-significant differences in the comparisons are not indicated.

dopamine found may thus represent precursor levels present during the biosynthesis of norepinephrine. This is supported by the findings that the patterns of regional differences and cyclic fluctuations in the tissue concentrations resemble those of norepinephrine.

Against the background of results obtained on rat uterus it can be assumed that the minute levels of epinephrine measured in the tube are not synthesized locally (Wurtman, Chu and Axelrod 1963a), but taken up from the circulation (Wurtman, Axelrod and Kopin 1963b, Wurtman, Axelrod and Potter 1964, Green and Miller 1966a) and then stored extraneuronally, e.g. diffusely in the smooth musculature (Wurtman et al. 1964). Since both the plasma epinephrine concentrations (Green and Miller 1966b) and the uterine binding of the amine (Wurtman et al. 1963b, 1964) change independently during the estrous cycle, an explanation is offered for the lack of consistent cyclic variations in the tube of the kind found for dopamine and norepinephrine. In view of the lack of evidence that epinephrine in the tube is synthesized and stored in special cells, the higher concentrations in the isthmus region may simply be related to a more efficient binding to the smooth musculature, which is more prominent here than in the ampulla and fimbria.

The highest concentration of norepinephrine in the isthmus, where the adrenergic nerves are primarily related to non-vascular smooth musculature, is consistent with the view that the musculature in this region may function as a sympathetically innervated sphincter mechanism. Although a tendency to a regional variation in the norepinephrine concentration was found in the isthmus, it did not provide sufficient evidence that the sphincter function would be restricted to any particular portion of the isthmus.

The norepinephrine concentration in both isthmus and ampulla was found to be higher in the luteal than in the follicular phase, confirming previous fluorescence histochemical and fluorometric observations in monkeys and humans (Dujovne et al. 1976; Sjöberg, Johansson, Owman, Rosengren and

Walles 1977). Thus, a high tubal norepinephrine concentration is associated with high plasma progesterone levels. A notable finding in the regional determinations of norepinephrine was that ovulation, presently defined as the stage when plasma estradiol concentration is maximal, is associated with a particularly high norepinephrine concentration in the isthmus, which is not the case in the ampullary region.

The differences in tubal norepinephrine concentration during the menstrual cycle could simply have reflected changes in tissue weight due to, e.g., altered water content. However, the present material did not reveal any significant cyclic variation in tissue wet weight, and the concentration of protein in the tube does not change to any significant extent (Batra, Helm, Owman, Sjöberg and Walles 1980). Therefore, the higher norepinephrine concentrations measured during the ovulatory and luteal phases represent a true increment in the neuronal transmitter level.

Measurements of norepinephrine in the rabbit oviduct have shown that estrogen treatment increases neuronal norepinephrine compared to untreated controls or animals receiving progesterone (Owman and Sjöberg 1976). Estrogen administration is associated with a more rapid turn-over of tubal noradrenalina than in rabbits receiving progesterone (Takeda and Doteuchi 1976), which would suggest that higher tubal norepinephrine levels reflect increased sympathetic nerve activity.

Exogenous norepinephrine is able to reduce tubal motor activity in vitro to a similar extent during both the follicular and luteal phases (Moawad, Hedqvist and Kim 1976; Owman, Falck, Johansson, Rosengren, Sjöberg, Sporrang, Svensson and Walles 1976; Helm, Owman, Sjöberg and Walles 1981). The uniformly higher transmitter level presently found in the tube during the luteal phase compared to the follicular phase may thus in functional terms suggest that the sympathomimetic inhibition of tubal motor activity in vivo is more prominent during the luteal phase. In line with this, the particularly high

norepinephrine concentration in the isthmus during ovulation, in combination with the observation that norepinephrine increases isthmic motor activity in vitro at this stage (Moawad et al. 1976; Owman et al. 1976; Helm et al. 1981), suggests a special functional significance of the adrenergic innervation in the isthmus during a limited period of the entire ovulatory process. This is consistent with the involvement of an adrenergic component in the tube locking mechanism, which appears to have crucial importance for normal fertilization and transport of the blastocyst towards the site of implantation (Pauerstein and Eddy 1979).

ACKNOWLEDGEMENT

Supported by grant No. 14X-5680 from the Swedish Medical Research Council, and from Förenade Liv Mutual Group Life Insurance Company, Stockholm, Sweden.

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Electron Microscopic and Pharmacological Evidence for a Functional Adrenergic
Innervation of the Smooth Musculature in the Human Fallopian Tube

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Electron microscopic and pharmacologic evidence for a functional adrenergic innervation of the smooth musculature in the human Fallopian tube. BRAIN RES. BULL. 0 (0) 000-000, 1981. — Triple fixation for electron microscopy of the isthmic region of the human Fallopian tube distinguished adrenergic nerve terminals through their content of synaptic vesicles with a diameter of 50-60 nm. Non-vascular adrenergic nerves were found both outside and within smooth muscle bundles. Adrenergic varicosities, partly devoid of their Schwann cell ensheathing, frequently approached the smooth muscle cells with a distance of about 200 nm, and sometimes as close as 60-100 nm. These close contacts often occupied a considerable area of the naked varicosity. Electrical field stimulation of adrenergic nerves in the isthmic smooth muscle preparations in vitro induced an alpha-receptor mediated, frequency-dependent contractile response. The results show that the sympathetic nerves in the human Fallopian tube are able to exert a motor control of its smooth musculature, and may thus be involved in local functions essential for the normal fertilization process.

Human Fallopian tube	Electron microscopy	Adrenergic nerves
Smooth musculature	Neuromuscular relationship	Transmural nerve stimulation
Contractile response		

INTRODUCTION

The smooth musculature of the Fallopian tube plays an important role in the transport of spermatozoa and ova, in fertilization, and in the transport of the blastocyst towards the uterus for implantation (19). There is reason to believe that a constrictory mechanism, possibly a sphincter, located in the ampullary-isthmic junction or in the isthmus, is responsible for the tube-locking process required to retain the ovum in the tube during fertilization (2). This sphincter-like region receives a well-developed adrenergic (17) and peptidergic (1) innervation, which may modulate hormonal effects on the smooth musculature through constrictory and relaxatory effects (5,9,10,13,15,17,18,22). There are considerable species differences in the neuromuscular functions of the tube, and only little is still known about basic properties of this organ in humans (8). The present study analyzes the adrenergic neuromuscular contacts and their functional significance in the human Fallopian tube.

MATERIAL AND METHODS

Material. The human tubal material was obtained from regularly menstruating women (29-51 years old) during abdominal hysterectomy (because of myoma), salpingo-oophorectomy (for benign ovarian cysts), and total or partial tubal resection (for sterilization). All patients were informed about the study and all gave their permission to use the tissue specimens. Only tubes with normal macroscopic appearance were used. Tissues from patients with suspected hormonal disturbances or receiving treatment with β -receptor antagonists or hormones were not included. Morphine chloride (10 mg) and scopolamine bromide (0.4 mg) were given as preanesthetic medication. Anesthesia was induced by pentothal sodium administered i.v., followed by inhalation of a mixture of dinitrous oxide and oxygen. Succinylcholine iodide was given initially i.v. as muscle relaxant, followed by pancuronium bromide.

Electron microscopy. Material was obtained from 13 patients. The tubes were exposed during laparotomy and a small longitudinal incision was made in the peritoneal covering of the mid-portion of the isthmus. About 15-20 mm of the isthmus was carefully dissected free from connective tissue without interfering with the blood supply. Ten mm of the exposed tube with its blood vessels was isolated between two clamps, rapidly dissected out, and pieces of about 1 mm^3 size were cut from the circular smooth muscle layer. The preparations were fixed in a modified, freshly prepared fixation solution (21) consisting of 1% glutaraldehyde and 0.4% paraformaldehyde in 0.075 M sodium chromate/potassium dichromate buffer (pH 7.2) at room temperature for 30-60 min. This was followed by immersion in buffer solution alone (pH 6.0) for 15 hrs and postfixation in 2% OsO_4 in the same buffer (pH 6.0) for 1 hr. The preparations were embedded in Vestopal W and sectioned on an LKB Ultratome III. Semithin sections were used for orientation in a light microscope, and ultrathin sections of silver-gold interference colour were examined and photographed in a Philips EM 300 electron microscope.

In vitro registration. Immediately after removal of the oviducts from 16 women, the adjacent peritoneal tissue was freed, the ampullary-isthmic junction was localized by the aid of a probe (2 mm o.d.), and the oviduct was divided so that the isthmic part could be used for recording of isometric smooth muscle activity in an organ bath. A 4 mm long cylinder from each isthmus was cut for registration of circular muscle activity, and a 6 mm long piece was used for longitudinal recordings. The pieces prepared for circular registration were mounted between two L-formed metal holders, and those for recordings of longitudinal smooth muscle activity were placed between two metal hooks using silk ligatures. Both preparations were connected to Grass FT03C force-displacement transducers, and their signals were amplified and recorded by a Grass model 7B polygraph. Electrical field stimulation was applied by two platinum electrodes located 10 mm apart on either side of

the preparations in the organ bath. Trains of biphasic pulses at supramaximal voltage (approximately 10V between the electrodes) were used at a frequency of usually 8 and 16 Hz, and at a low pulse duration (1 msec) with the aim of stimulating the nerves selectively, without directly activating the smooth musculature in the preparations (16).

The organ baths contained a modified Krebs-Ringer buffer solution of the following composition (concentrations in millimoles): NaCl 118, KCl 4.5, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 2.5, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 1.0, NaHCO_3 25, KH_2PO_4 1.0, glucose 6.0. The temperature of the baths was thermostatically maintained at 37°C, and the buffer solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 giving a pH of 7.40 (range 7.25 to 7.55). All preparations were given an initial passive tension of 500 dynes and were allowed to accommodate for 45-60 min. During this period the tension decreased by 150-200 dynes and the preparations started to contract spontaneously. The alpha receptors were blocked by phentolamine given into the bath in a final concentration of 3×10^{-6} - 10^{-5} M. In some experiments the nerve blocking agent tetrodotoxin (10^{-7} - 10^{-6} M) was used.

RESULTS

Electron microscopy. Non-myelinated nerves were found in bundles enclosed by a Schwann cell sheath (Fig. 1) and running in the connective tissue between the smooth muscle layers. Closer to the smooth muscle cells forming the circular layer, the Schwann cell ensheathing was partly broken up, and axon varicosities characterized by numerous synaptic vesicles and mitochondria were seen (Fig. 2). The adrenergic type of varicosities was identified by the presence of highly electron-dense, 50-60 nm vesicles, together with vesicles of 100 nm diameter with a moderately dense content. Membrane-bound structures with a highly electron-dense core sometimes had a biconcave form (Fig. 3). The naked part of the adrenergic nerve varicosities often approached

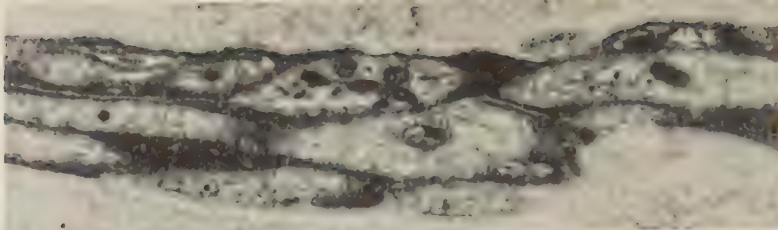


Fig. 1. Bundles of non-myelinated nerves enclosed by a Schwann cell sheath running in the connective tissue. X 29,000.

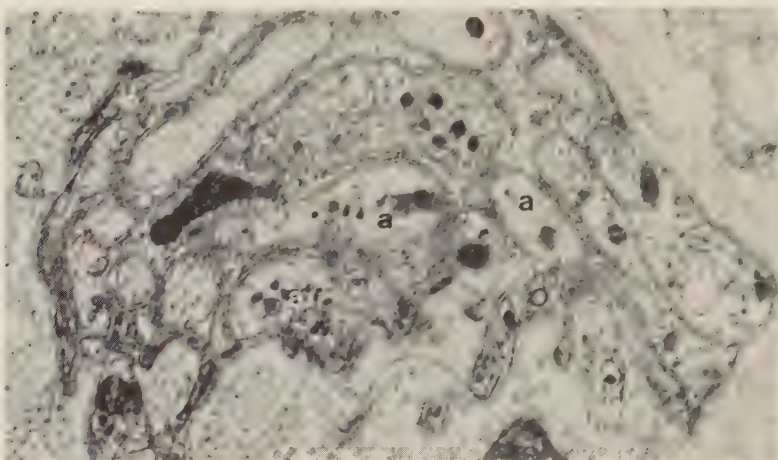


Fig. 2. Several axons and axon varicosities enclosed by incomplete Schwann cell sheath. Such nerve bundles are found near the smooth muscle cells. The adrenergic terminals (a) are characterized by electron-dense synaptic vesicles of 50-60 nm diameter. X 32,000.

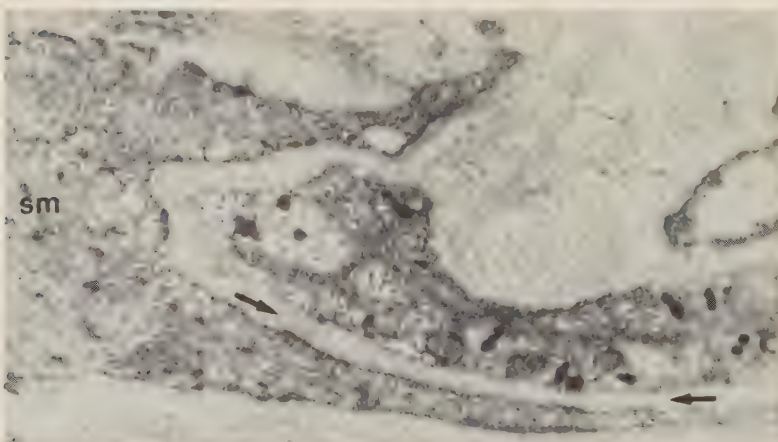


Fig. 3. Adrenergic nerve terminal located at 70 nm distance from the membrane of a smooth muscle cell (sm). The apposing membrane of the terminal is not covered by the Schwann cell. The size of the contact area (between arrows) is considerable. X 35,000.

the smooth muscle cells with a distance of approximately 200 nm, and sometimes the axon membrane came as close as 60-100 nm from the membrane of the muscle cell. These close contacts, containing only the basement membranes, usually occupied a considerable area of the naked varicosity (Fig. 3), though membrane specializations were not seen either on the axonal or on the muscular side. The smooth muscle cells frequently had an irregular contour, with long slender foldings (Fig. 3), suggesting that they to a large extent were in a contracted state.

In vitro registration. During the process of accommodation the preparations started to contract spontaneously (Fig. 4). Electric field stimulation resulted in an initial twitch contraction, with an amplitude clearly higher than that of the individual peaks in the phasic contractions present before stimulation (Fig. 4a). Immediately following the twitch contraction the tube relaxed, often in combination with a reduced or abolished phasic activity. Following a 1-min stimulation period the preparation returned to its normal activity in the course of another 1-2 min. This pattern of response was almost equal for the circular and longitudinal smooth muscle preparations of the isthmus. The twitch contraction was frequency-dependent, with the highest amplitude at 8-16 Hz (Fig. 5). In order to ascertain that the reduction in the response at high frequencies was not simply due to muscular fatigue, the frequency series was completed by renewed stimulation at 16Hz, which produced the same amount of contraction as during the previous test with increasing frequencies.

Twenty min after addition to the bath of the alpha-receptor antagonist, phentolamine, the twitch contraction during electric field stimulation was abolished (Fig. 4b). The relaxation following the twitch was not overtly altered. The twitch contraction during transmural electrical stimulation was also abolished in the presence of tetrodotoxin, which blocks the nerves by inhibiting the Na^+/K^+ transport across the axonal membrane. Tetro-

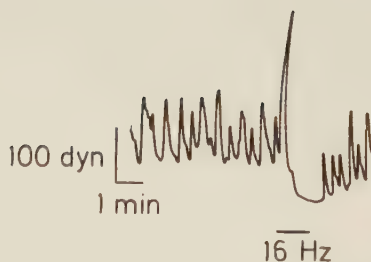


Fig. 4a

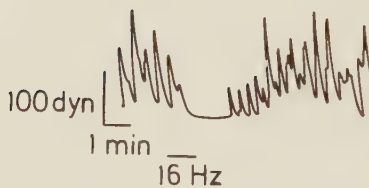


Fig. 4b



Fig. 4c

Fig. 4. Transmurial nerve stimulation of spontaneously contracting circular smooth muscle of the tubal isthmus. (a) Twitch contraction followed by relaxation during 16 Hz stimulation. (b) Phentolamine ($3 \times 10^{-6} \text{ M}$) abolishes the contractile twitch without affecting the relaxation. (c) Tetrodotoxin (10^{-6} M) blocks the response during electrical stimulation without affecting the phasic motor activity.

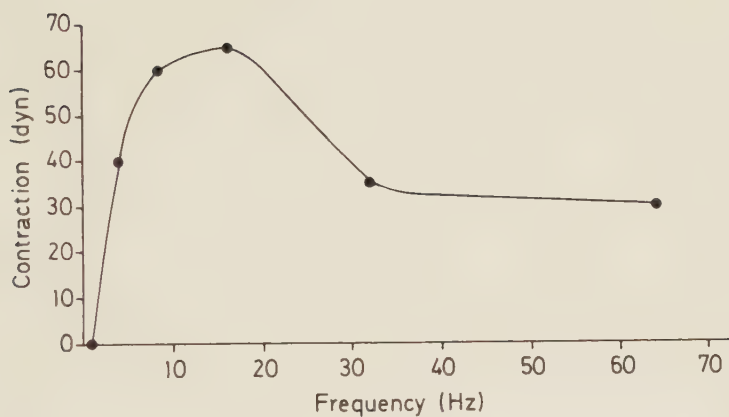


Fig. 5

Fig. 5. Representative experiment showing frequency-response relationship of the contractile response in the circular smooth musculature of isthmus during electrical field stimulation of the nerves (10 V, 1 msec pulse duration).

dotoxin also eliminated the relaxation following the twitch, but the phasic motor activity was not affected.

DISCUSSION

Using the "triple fixation" technique (21) for electron microscopy it is possible to distinguish the adrenergic nerve terminals through their content of electron-dense synaptic vesicles of 50-60 nm diameter. In the human tubal preparations, adrenergic nerves were identified both outside and within smooth muscle bundles. This confirms the electron microscopic observations on the human Fallopian tube by Ishii (12). A similar organization of the adrenergic nerves has been described for the rabbit (11) and rat (12). On the other hand, Daniel et al. (7) claimed that nerves in the human tube are primarily present in the peripheral regions of the musculature, which contains chiefly collagen and blood vessels.

In the present study, the adrenergic nerve terminals of the isthmus were often found to be located approximately 200 nm from the membrane of (non-vascular) smooth muscle cells in the form of simple appositions. The Schwann cell ensheathing was incomplete with exposed parts of the nerves facing the smooth musculature. This type of cell contacts has been found also in the rabbit (11) and rat (12) oviducts. Sometimes we found the neuromuscular contacts to be as close as 60-100 nm, occupying a considerable area of the naked varicosity. Thus, it appears that the isthmus of the human Fallopian tube is better equipped with adrenergic neuromuscular contacts than previously thought (7). Although the minimum neuromuscular distance in many smooth muscle tissues is 80-120 nm, transmitter released from nerves located 300-1000 nm or more away from smooth muscle cells is likely to be effective (3,6), which has been particularly well elucidated in sympathetically innervated vascular smooth musculature (4,14). Thus, the released noradrenaline probably reach the smooth muscle cells through

diffusion, followed by myogenic spread of electrical activity by way of close appositions (rather than nexus formations) between the muscle cells (7,11). These cell-to-cell contacts also join smooth muscle cells in different layers (7), which means that activity originating in those muscle cells being directly supplied with adrenergic nerve terminals will spread throughout the isthmus involving not only the circular but also the longitudinally oriented muscle fibres.

The ultrastructural observations were supported by the pharmacological experiments in vitro, showing that electrical stimulation of the nerves releases sufficient amount of noradrenaline to produce a frequency-dependent motor response in the isthmus. The experiments show that the nervous system exerts its full range of alpha-receptor mediated smooth muscle contraction at low impulse frequencies. The reduction in the contractile response during more intense nerve activation is probably due to failure of the nerve endings to release the transmitter at high frequencies (20). Since the present study deals only with the adrenergic component in the autonomic innervation of the Fallopian tube, the motor effects of nerve stimulation has been focused on the contractile response: nerve-induced reduction in tubal motor activity is more complex, involving also e.g. peptidergic nerves (1,9,22) in addition to beta-adrenergic mechanisms (10,13,15,17,18).

Our results on the ultrastructural appearance of the neuromuscular contacts in the human tubal isthmus, in combination with the alpha-receptor mediated motor effects of transmural nerve stimulation, are consistent with a role of adrenergic nerves in modifying contraction of the organ.

Acknowledgement. Supported by grant No. 14X-5680 from the Swedish Medical Research Council and from Förenade Liv Mutual Group Insurance Company, Stockholm, Sweden.

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Motor activity of the human Fallopian tube in vitro in relation to plasma concentration of oestradiol and progesterone, and the influence of noradrenaline

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Summary. The contractile pattern of the human Fallopian tube was studied in preparations from the ampullary and isthmic regions mounted in an organ bath for measurement of longitudinal and circular smooth muscle activity. The material was obtained during the follicular, ovulatory, and luteal phases of the cycle as defined primarily from plasma oestrogen and progesterone values. The frequency of the spontaneous contractions increased progressively during the follicular phase to become maximal around ovulation. There was no consistent difference between isthmus and ampulla; the circular musculature had a higher frequency than the longitudinal during the ovulatory phase. Noradrenaline ($3 \times 10^{-6}M$) in general potentiated the difference in frequency seen between the ovulatory phase on the one hand and the follicular and luteal phases on the other. Contractile activity, assessed by integrating the curve on the pen-recorder trace by using a planimeter, increased markedly during the ovulatory phase in all types of smooth muscle preparations. Exogenous noradrenaline inhibited spontaneous motor activity in preparations from pregnant or post-menopausal women or from women taking combined-type oral contraceptives. This effect was most marked in the circular muscle. Thus the different regions of the human Fallopian tube in vitro show various patterns of spontaneous motor activity in relation to the plasma steroid concentrations during the menstrual cycle. Responses to exogenous noradrenaline also varied, indicating that the effects of endogenous noradrenaline released from sympathetic nerves may vary similarly.

Introduction

The Fallopian tube is engaged in various transport processes essential for reproduction (see Pauerstein & Eddy, 1979). Within the ampullary region the egg is not transported in a simple continuous pattern, but makes a complex to-and-fro movement providing a net forward transport velocity in the order of 10 mm/min (Verdugo, Blandau, Tam & Halbert, 1976). This is probably governed by a combination of smooth muscle activity and the motility of cilia, resulting in an assymetric frictional effect (Halbert, Tam, Adams & Blandau, 1976; Eddy, Flores, Archer & Pauerstein, 1978). In women, egg transport is characterized by a retention in the ampulla, near the ampullary-isthmic junction, for 70-80 h followed by a rapid transit through isthmus in the course of less than 10 h (Cheviakoff et al., 1976; Croxatto et al., 1978). This tube-locking mechanism, as well as the rapid passage into the uterus, seems to be effected primarily by tubal muscular activity (Anand & Guha, 1978). In most species investigated, including man, the overall time required for the transport of the ovum from the ovary to the uterus is 3-4 days (Pauerstein & Eddy, 1979). There is strong evidence that the tubal motor activity is influenced by local adrenergic neural mechanisms (Brundin, 1965; Owman, Rosengren & Sjöberg, 1967).

The timing of the different components in the complex process of ovum transport is under the influence of sex hormones (for references, see Harper et al., 1976). Most of the available information has been obtained from laboratory animals and, although much has been learnt about tubal motility in women from recordings by implanted pressure transducers, little is known about the mechanisms controlling tubal transport functions. The present study on isolated tubal tissues

from women was undertaken to gain information about smooth muscle activity, and the total response to the sympathetic neurotransmitter, noradrenaline, during the different phases of the menstrual cycle.

Materials and Methods

Material

The human tubal material was obtained by abdominal hysterectomy (because of adenomyosis, myoma, or preinvasive carcinoma of the cervix), salpingo-oophorectomy (for benign ovarian cysts), and salpingectomy (because of sterilization). One or both Fallopian tubes were taken from 86 adult, regularly menstruating patients aged 30-48 years. Tubal material was also obtained from 7 women (aged 31-47 years) taking contraceptive pills of the combined steroid type (50 µg ethinyloestradiol plus 0.25 - 3 mg synthetic gestagen), 3 pregnant patients (aged 30-41 years) in the first trimester, and 6 menopausal women (aged 46-58 years). Only tubes with normal macroscopic appearance were used. Tissues from patients with suspected hormonal disturbances or receiving treatment with β -receptor antagonists or hormones (except for contraceptive pills as above) were not included.

Morphine chloride (10 mg) and scopolamine bromide (0.4 mg) were given as preanaesthetic medication. Anaesthesia was induced by pento-barbitone sodium administered i.v., followed by inhalation of a mixture of dinitrous oxide and oxygen. Succinylcholine iodide was given initially i.v. as muscle relaxant, followed by pancuronium bromide.

Definition of hormonal state

The cyclic stage was estimated on the basis of (1) the patient's menstrual cycle as noted in the record, usually together with histological examination of endometrial material obtained by curettage of the uterine cavity during the operation, (2) sectioned and stained endometrial preparations and (3) plasma steroid levels.

Blood (approximately 10 ml) was withdrawn from a vein within 1 h before opening of the abdomen for determination of progesterone and oestradiol concentrations in plasma. The progesterone radioimmunoassay was similar to that described by Youssefnejadian, Florensa, Collins & Sommerville (1972). The antiserum (FO 22-5-73), which was highly specific for progesterone, was supplied by Dr Kjell Martinsson (Stockholm) and was used at a dilution of 1:1500. The antiserum was raised in sheep against BSA conjugated with 11α -hydroxyprogesterone. Usually 25 - 100 μ l plasma were extracted twice with petroleum ether. The unbound steroid was removed by addition of dextran-coated charcoal. The sensitivity of the assay was 25 pg/ml and the inter- and intra-assay coefficients of variation were 18.7 and 10.5 %. Cross-reaction of the antiserum was < 10 % to 17α -hydroxyprogesterone and < 0.05 % to 20α -hydroxyprogesterone. The radioimmunoassay for oestradiol was that described by Lindberg, Lindberg, Martinsson & Johansson (1974). The antiserum was raised in sheep against oestradiol- 17β -oxime conjugated with BSA and used at a dilution of 1:100 000. Extractions of 1 ml plasma were with diethyl ether. The unbound steroid was removed by addition of dextran-coated charcoal. The assay sensitivity was 5 pg oestradiol/ml and the inter- and intra-assay coefficients of variation were 16.2 and 9.9 %. Cross-reaction of the antiserum to oestradiol- 17α was 0.7 %, and it was 11 % for oestrone (Batra,

Bengtsson & Sjöberg, 1979; Batra, Öwman, Sjöberg & Thorbert, 1979).

The plasma hormone concentrations were used to confirm the separation of the material into three main stages, based on the patient's menstrual record and the histological examinations:

	Progesterone	Oestradiol
Follicular phase	≤ 2 ng/ml	≤ 100 pg/ml
Ovulatory phase	≤ 2 ng/ml	> 100 pg/ml
Luteal phase	> 2 ng/ml	any value

Tubal preparations

Immediately after removal, the oviducts were placed in ice-cold Krebs-Ringer buffer solution (see below) for < 1 h or for as long as 20 h (at 4°C). Pilot studies on tissues before and after storage from the same patients have shown no change in motor functions. Before mounting in the organ bath, a thin (2 mm o.d.) probe was introduced into the lumen from the ampullary end to localize the ampullary-isthmic junction, where the oviduct was divided. A few mm of the adjacent isthmic and ampullary portions were discarded. The next 10 mm of the two regions were used for recordings of isometric smooth muscle activity. A 4 mm long cylinder was cut and mounted for registration of mainly circular activity, and the remaining 6 mm long piece was used for recordings of mainly longitudinal smooth muscle activity. The identity of the ampullary and isthmic preparations was confirmed by microscopy of haematoxylin and eosin-stained sections. To estimate the thickness of the various layers of the tubal wall, avoiding as far as possible shrinkage artefacts, adjacent pieces of ampulla and isthmus were frozen to -80°C , sectioned in a cryostat at $20\text{ }\mu\text{m}$ thickness, stained with haematoxylin and eosin, and measured in a microscope.

The pieces prepared for circular activity measurements were mounted between two L-shaped metal holders in organ baths. The pieces for longitudinal recordings were tied to two metal hooks by silk ligatures. All four preparations were connected to Grass FT03C force-displacement transducers, and their signals were amplified and recorded by a Grass Model 7B Polygraph. The organ baths contained a modified Krebs-Ringer solution of the following composition (mmol): NaCl 118, KCl 4.5, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 2.5, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 1.0, NaHCO_3 25, KH_2PO_4 1.0, glucose 6.0. The temperature of the baths was thermostatically maintained at 37°C, and the buffer solution was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2 giving a pH of 7.4 (range 7.25 to 7.55). All preparations were given an initial passive tension of 500 mg force and were allowed to accommodate for 45-60 min. During this period the tension decreased and the preparations started to contract spontaneously.

Analysis of data

Noradrenaline (L-artenerol hydrochloride, Sigma; dissolved in 0.9 % w/v NaCl with the addition of 0.2 mg/ml ascorbic acid as antioxidant) was usually given as one standard dose (3×10^{-6} M final concentrations in the organ bath), but the action of increasing doses was also tested during cumulative application (see also Helm, Owman, Sjöberg & Walles, 1981). The frequency of the spontaneous contractions was measured during one 3-min period immediately before administration of the amine and compared to the frequency of contractions during an equal period just after the administration. Frequency determinations in the more regularly contracting circular preparations did not present any difficulties. The longitudinally

orientated preparations sometimes contracted irregularly with individual deflections of variable size. All clearly distinct deflections were included in the frequency estimations. The degree of increase or reduction in activity was obtained by using a planimeter to integrate the curves over the 3-min periods. The base line was defined as the lowest level of tone from which the individual deflections originated during the total 6-min measurement period. The noradrenaline-induced change in activity of the preparations was often associated with alterations in the amplitude of the individual rhythmic contractions, but was too inconsistent to be useful.

Mean values (and standard errors) of the various parameters of motor activity in the different types of smooth muscle preparations were related to the phase of the menstrual cycle and differences were compared by Student's t test. Attempts were also made to obtain a simultaneous correlation of individual values for plasma steroid hormones and the type of contraction in a three-dimensional system covering the steroid levels of the entire menstrual cycle. The original values for the recorded change in smooth muscle activity were plotted in a two-dimensional square grid where the plasma levels of oestradiol and progesterone were represented in the x and y axes. Each square is assigned a value equal to the average of the observation inside the square. As the observation points are placed very irregular in the examined area, some squares did not include any observation. The values of these squares were approximated from the closest observations of adjacent squares using three different interpolation methods: double-linear interpolation, quadratic interpolation, and distance-weighting interpolation (Bladh & Jern, 1980). The relationship between the regular values obtained for the motor response and the plasma steroid levels were then depicted with the

use of a three-dimensional colour ink jet plotter (Smeds, 1973) available at the Computer Center of the University of Lund where the diagrams were devised in collaboration with Mikael Jern and Lars Persson.

Results

As shown in Text-fig. 1, there was no consistent difference in contraction frequency between the isthmic and ampullary preparations, and the circular and longitudinal muscles differed only in the isthmus ($P < 0.01$) and at the time of ovulation.

An over-all picture of the cyclic variations in the frequency of the spontaneous contraction was obtained with the three-dimensional computer plots. Text-figure 2 exemplifies the circular muscle and shows that there was a steady increase in frequency during the follicular phase (increasing oestradiol and low progesterone) towards a maximum, which was reached around the time of ovulation (i.e. highest oestradiol values, but low progesterone), followed by a return to a lower frequency in the course of the luteal phase (increasing progesterone levels). This pattern of changes was similar in the circular musculature of the isthmus and the ampulla. There were no such consistent cyclic changes in frequency of the longitudinally orientated tubal preparations.

The menopausal patients as well as those taking steroid-type contraceptives showed little individual variation in plasma steroid levels; progesterone concentration was low, and the mean oestradiol value corresponded to that expected during early and late follicular phase, respectively (Table 1). There was no statistically significant difference in the frequency of the spontaneous contractions of the various types of preparations in these two groups compared with

Table 1. Absolute frequency of spontaneous contractions, and % change in area under the curves of motor activity induced by 3×10^{-6} M-noradrenaline in comparison with the condition before treatment, in four types of human oviduct smooth muscle preparations (increased activity corresponds to positive values, reduced activity to negative values). Preoperative plasma values of oestradiol (E_2) and progesterone (P) are given.

Plasma steroids (E ₂ :pg/ml; P: ng/ml)	Measurement	Isthmus		Ampulla	
		Circular	Longitudinal	Circular	Longitudinal
Menopause (n = 6)					
E ₂ = 38.7 ± 6.3	Frequency [†]	10.7 ± 1.2	8.3 ± 0.1	12.0 ± 1.6	10.2 ± 1.4
P = 0.4 ± 0.2	% change in area	-12.3 ± 3.1**	-6.7 ± 9.8	-10.5 ± 4.3*	-2.7 ± 6.2
Steroid contraceptives (n = 7)					
E ₂ = 70.7 ± 17.5	Frequency [†]	11.4 ± 2.2	9.2 ± 1.2	9.3 ± 1.9	11.7 ± 1.7
P = 0.4 ± 0.1	% change in area	-21.7 ± 7.0**	12.0 ± 11.0	-19.0 ± 5.1***	-0.7 ± 10.3
Pregnancy (n = 3)					
E ₂ = 746.7 ± 382.9	Frequency [†]	6.7 ± 1.2	6.3 ± 2.4	5.3 ± 0.3	6.0 ± 1.0
P = 21.2 ± 8.7	% change in area	-29.3 ± 10.4	-35.0 ± 9.3*	-20.7 ± 10.9	-36.0 ± 8.6*

Values are mean $\pm$ s.e.m. for the no. of preparations in parentheses

Paired t-test comparing the motor activity before and after noradrenaline administration: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[†]The absolute frequencies of spontaneous tubal contraction in normally menstruating women during various cyclic stages

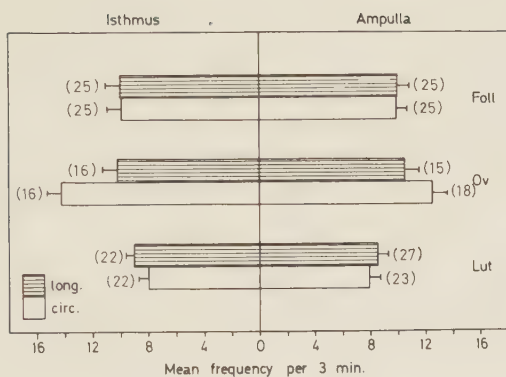


Fig. 1.

Text-fig. 1. Frequency of spontaneous tubal contractions (isthmus and ampullary longitudinal and circular musculature) during the follicular, ovulatory and luteal phases of the menstrual cycle. Values are means + s.e.m. for the no. of preparations in parentheses.

preparations taken in the follicular phase of normally menstruating women (Text fig. 1). The contraction frequency in early pregnancy was lower than that during the luteal phase of the cycle but was significantly different only for the ampullary preparations ($p < 0.05$).

Noradrenaline lowered the contraction frequency of both the isthmic and the ampullar circular preparations in the follicular and luteal phases, but increased that of the longitudinal ampullar preparation in the ovulation period. The mean effect of noradrenaline on the amplitude of the response (i.e. the size of the deflections above the tonic activity) was usually a reduction in all phases and in all types of tissues, although the effects were very variable and no statistically significant difference was apparent.

The noradrenaline-induced changes in surface area under the curves of motor activity are given in Table 2 and the planimetric results for isthmic circular muscle in Text-fig. 3. Contractile activity was markedly increased around ovulation in all types of muscle preparation, and in the longitudinal isthmus in the follicular phase. Significant reductions occurred during the luteal phase in the circular muscle of isthmic and ampullar preparations and in the follicular phase for the circular isthmus preparation.

In the menopausal, contraceptive-taking and pregnant women (Table 1) noradrenaline always reduced the contractile activity (i.e. the surface area under the curve) in the circularly orientated musculature. The individual responses in the longitudinal musculature varied between slight reduction and enhancement in activity, except during pregnancy when there was a more consistent pattern of reduction also in the longitudinally orientated tubal smooth musculature.

Table 2. Percentage changes of motor activity induced by 3×10^{-6} M-noradrenaline in four types of human oviduct smooth muscle at different phases of the menstrual cycle (increased frequency/area indicated by positive values, reduced frequency/area by negative values)

Stage of cycle	Isthmus		Ampulla	
	Circular	Longitudinal	Circular	Longitudinal
	Frequency of phasic contractions			
Follicular	-11.5 $\pm$ 6.1 (23)*	-5.5 $\pm$ 4.1 (26)*	-15.9 $\pm$ 6.5 (25)**	4.6 $\pm$ 4.3 (25)
Ovulatory	2.6 $\pm$ 4.6 (19)	-0.6 $\pm$ 7.0 (16)	2.9 $\pm$ 7.9 (18)	15.5 $\pm$ 5.5 (15)***
Luteal	-8.6 $\pm$ 10.2 (23)	-9.1 $\pm$ 6.2 (22)	-7.0 $\pm$ 8.5 (23)	-6.2 $\pm$ 6.9 (27)
Area under the recorded curve				
Follicular	-18.8 $\pm$ 5.4 (24)**	37.7 $\pm$ 24.9 (27)*	-11.8 $\pm$ 7.2 (24)	11.2 $\pm$ 7.8 (25)
Ovulatory	51.2 $\pm$ 27.2 (18)	198.1 $\pm$ 90.1 (15)	17.6 $\pm$ 5.5 (18)*	51.8 $\pm$ 19.7 (15)*
Luteal	-33.9 $\pm$ 4.4 (23)**	3.7 $\pm$ 13.0 (24)	-18.4 $\pm$ 6.9 (23)*	-15.5 $\pm$ 8.5 (27)

Values are mean $\pm$ s.e.m. for the no. of preparations given in parentheses.

Values significantly different from those before noradrenaline treatment (paired t test):

* $\bar{p} < 0.05$; ** $\bar{p} < 0.01$; *** $\bar{p} < 0.001$.

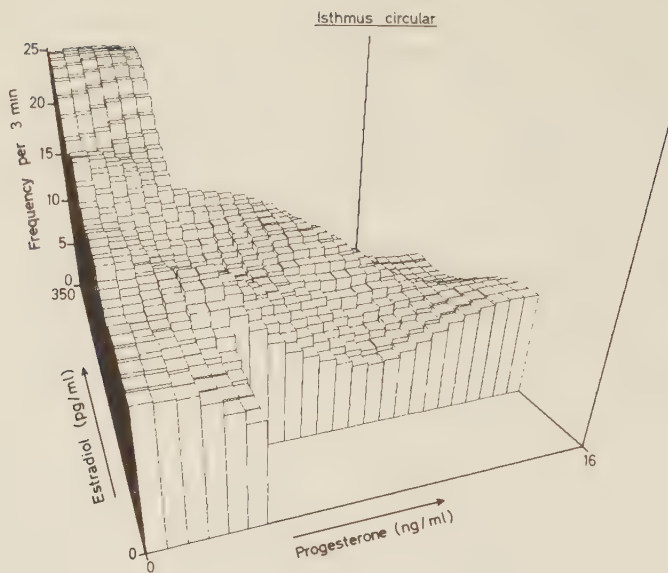


Fig. 2.

Text-fig. 2. Three-dimensional computer plot, based on the entire material from the circularly orientated smooth musculature of the isthmus ($\bar{n} = 63$), illustrating the frequency of spontaneous contractions in relation to the plasma levels of oestradiol and progesterone. The original, irregular frequency values have been transformed into regularity by computerized interpolation (see text).

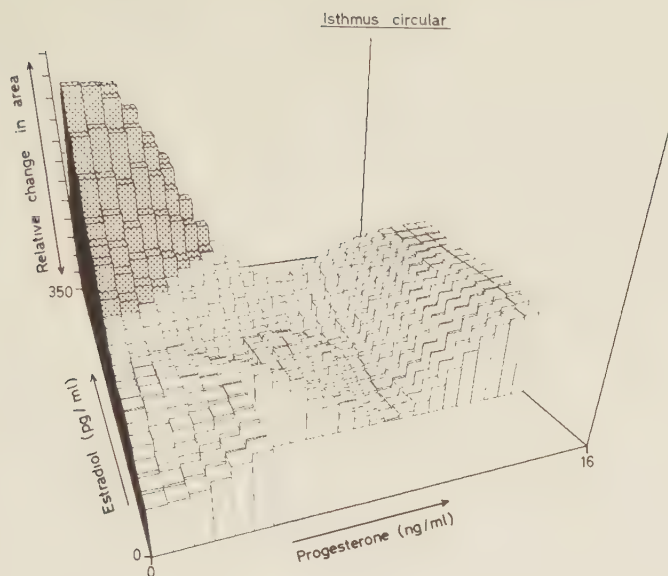


Fig. 3.

Text-fig. 3. Three-dimensional computer plot, based on the entire material from the circular isthmus smooth musculature ($n = 63$), showing increase (shaded columns) or decrease (open columns) in area under curves of motor activity induced by 3×10^{-6} M-noradrenaline, in relation to the plasma levels of oestradiol and progesterone. The relative changes in area were determined planimetrically during equal time-periods before and after administration of the amine. The original, irregular values for the area changes thus obtained were transformed into regularity by computerized interpolation (see text).

Discussion

Most of our knowledge about tubal motor activity and its relationship to ovum transport is based on animal studies, particularly using rabbits (Harper et al., 1976). Despite intensive research, surprisingly little is understood about the significance of tubal motor functions in the pick-up, retention, and transit mechanisms for the ovum (Pauerstein & Eddy, 1979). Much information about the tubal motor activity in women has been gained from pressure recordings in vivo, but these have a number of practical and ethical limitations and are complicated to standardize and evaluate appropriately for quantitative pharmacological analysis (see Maia & Coutinho, 1976; Guiloff-Fische, Ibarra-Polo & Gómez-Rogers, 1976). Basic properties of the contractile functions of the human Fallopian tube have therefore been studied under precise conditions in vitro in combination with information of the patient's endocrine state (see also Moawad, Hedqvist & Kim, 1976; Öwman et al., 1976; Paton, 1976).

Lindblom, Hamberger & Wqvist (1978) used carefully microdissected and prepared smooth muscle elements from the circular and longitudinal musculature so that the separate types of smooth muscle were accurately defined in terms of their histological orientation, and contamination with blood vessels was to a large extent avoided. A disadvantage is that the complex architecture of the smooth musculature and its relationship to stromal elements in the tube, different for different segments of the organ, is disrupted. In the preparation type used in the present study, the wall of the tube is kept intact except for its connective tissue covering, but the activity is measured principally in the circularly or longitudinally orientated muscle layer. The quantitative contribution of vascular smooth musculature is probably small. The size of the preparations used (0.7 mm muscle

thickness) is unlikely to constitute a factor influencing oxygenation or diffusion (Helm et al., 1981).

The various patterns of contractile activity were related to different phases of the menstrual cycle on the basis of plasma oestradiol and progesterone determinations (Mishell et al., 1971). The 'ovulatory phase' samples may be a heterogeneous group, consisting not only of material from women in the actual process of ovulation, but also of material from the late follicular and probably also very early luteal stages. Better separation was not possible without access to LH peak data, and the patient's own menstrual cycle anamnesis was not considered sufficiently reliable for further sub-grouping of the material.

In general, the frequency, the pattern of cyclic variations in frequency, and the difference between the circular and longitudinal smooth musculature in the composite type of preparations that we used were very similar to those recorded for microdissected smooth muscle preparations from the human tube by Lindblom, Hamberger & Ljung (1980). The results for women taking combined steroid-type oral contraceptives, and menopausal and pregnant women agree with what could be expected on the basis of the relation found between plasma steroid levels and contraction frequency in patients with normal menstrual cycles (i.e. receiving no contraceptive pills).

The effect of noradrenaline on tubal motor activity was studied to illustrate the net response that might be expected following sympathetic neurotransmitter release in the tube at different cyclic stages. A standard dose of noradrenaline ($3 \times 10^{-6}M$) was chosen which, in a separate study (Helm et al., 1981), produced an α -receptor-mediated effect between the half maximum and maximum response. This concentration in the region of the receptor is in the same

order of magnitude as the estimated instantaneous concentration of noradrenaline occurring in the synaptic cleft of a neuromuscular contact during the passage of a sympathetic nerve impulse (Bennett, 1972). Noradrenaline administration into the organ bath potentiated the difference in frequency of contractions during the ovulatory phase in comparison with the follicular and luteal phases. However, the contraction frequency represents only an isolated aspect of tubal motor activity. Planimetric measurement of the surface area under the recorded curve before and after the noradrenaline administration confirmed that an α -receptor-mediated sympathomimetic increase in motor activity prevails during ovulation, whereas the contribution by a β -receptor mediated reduction in motor activity is more prominent during the follicular and ovulatory phases (Moawad et al., 1976; Helm et al., 1981).

There is ample evidence from studies on various laboratory animals that tubal motor activity is influenced by sex steroids, oestradiol being associated with increased tubal contractility, whereas progesterone primarily seems to have an inhibitory effect (Spilman & Harper, 1974; Borda, Sterin-Borda, Gimeno, Sterin-Speziale & Gimeno, 1975; Gimeno, Borda, Sterin-Borda, Sterin-Speziale & Gimeno, 1976). The biological activity of these steroids is probably governed by their concentrations in the tissue rather than in blood. Although there is a similarity in the general pattern of the cyclic fluctuations in the mean steroid concentrations between tubal tissue and plasma, the correlation between these values in each individual patient is poor (Batra, Helm, Owman, Sjöberg & Waller, 1980). The consistently high tubal motor activity, spontaneous and after sympathomimetic stimulation, found in the present in vitro study corresponds with high progesterone receptor concentration in the tissue

(Batra et al., 1980). Following ovulation, when the plasma progesterone levels rise, there is a drop in the specific tissue binding of progesterone which is associated with a reduced motor activity in the oviduct following noradrenaline administration. The effect of noradrenaline is less consistent, particularly in the longitudinal smooth musculature, during the follicular phase. This may reflect a progressive increment in α -adrenoceptor activity in relation to the β -receptors during the follicular phase (Moawad et al., 1976), when there are high concentrations of oestradiol and progesterone receptors (Robertson, Landgren & Guerrero, 1975; Batra et al., 1980; Verhage, Akbar & Jaffe, 1980).

The human Fallopian tube receives a well-developed adrenergic nerve supply (Brundin & Wirsén, 1964; Owman et al., 1967). The innervation is particularly dense in the isthmic region. Cyclic variations have been demonstrated in the levels of the sympathetic neurotransmitter in the organ (Owman et al., 1976; Sjöberg, Johansson, Owman, Rosengren & Waller, 1977) and there is reason to believe that this reflects a steroid-induced effect on parameters such as the functional turnover of noradrenaline in the tubal nerves (Takeda & Doteuchi, 1976). The interaction between sex steroids and sympathetic nerve function is characteristic for the reproductive tract (Alm, Björklund, Owman & Thorbert, 1979; Thorbert, Alm, Owman & Sjöberg, 1979), where it seems to be an important component in the function of the entire neuroeffector system (for review, see Thorbert, 1978). We therefore believe that the presence of oestradiol and progesterone can control motor activity of the tubal smooth musculature per se, can influence the way this smooth musculature, via the adrenoceptors, responds to the sympathetic neurotransmitter, and also can affect the function of the tubal adrenergic nerves themselves.

This work was supported by the Swedish Medical Research Council (grant No. 14X-5680) and from Maggie Stephens' foundation. We are deeply grateful to Mrs Barbro Gustavsson for skilful technical assistance.

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Table 2. Values for the dissociation constant (K_D) of the receptor-antagonist (phentolamine) complex in the phenylephrine-induced contraction of various types of tubal smooth muscle.

The values (mean $\pm$ S.E.M.) were grouped to represent two parts of the menstrual cycle (n = number of preparations tested): the follicular phase, with low plasma estradiol and progesterone levels; and the remainder of the cycle, having moderate to high plasma steroid levels. Differences between the mean values in the two groups were evaluated in Student's t-test; n.s. = non-significant.

Plasma steroid concentration	Isthmus		Ampulla	
	Circular	Longitudinal	Circular	Longitudinal
Low	$(6.82 \pm 1.73) \times 10^{-8}$ n = 14 p < 0.05	$(6.37 \pm 0.72) \times 10^{-8}$ n = 43 n.s.	$(5.46 \pm 1.70) \times 10^{-8}$ n = 8 p < 0.01	$(15.36 \pm 2.67) \times 10^{-8}$ n = 22 p < 0.05
Moderate to high	$(24.08 \pm 7.30) \times 10^{-8}$ n = 18	$(5.76 \pm 0.44) \times 10^{-8}$ n = 44	$(16.26 \pm 3.97) \times 10^{-8}$ n = 9	$(8.98 \pm 1.12) \times 10^{-8}$ n = 23

$F_{2\alpha}$ (2.5 - 250 ng/ml) excluding simple technical failure in these instances. The preparations responding to phenylephrine showed a marked variability in the degree of contraction, from some 30 dynes to 300 dynes.

The K_B values for the complex between phentolamine and the alpha-receptor appeared to represent two populations: one with values around 7×10^{-8} M and one around 2×10^{-7} M. The values of K_B in these two populations seemed to be related to cyclic stages characterized by, on the one hand, low plasma estradiol and progesterone concentration (i.e. the follicular phase), and moderate to high steroid values, on the other (i.e. around ovulation and in the luteal phase), as summarized in Table 2. Comparison of the mean K_B values between these two groups of preparations showed a statistically significant difference in the circular smooth musculature, both in isthmus ($p < 0.05$) and ampulla ($p < 0.01$). The K_B values in the longitudinal muscle were significantly different in the ampulla ($p < 0.05$) but not in the isthmus.

DISCUSSION

The human Fallopian tube is characterized by a complex pattern of motor activity demonstrable both in vivo and in vitro (for references, see Harper et al. 1976). It shows spontaneous contractions, which seem to require an intact prostaglandin synthesis (Tonpe & Lindblom 1979). The smooth musculature of the tube receives a well-developed autonomic innervation (Owman et al. 1967, Moawad et al. 1977, Helm et al. 1981 b and c). Noradrenaline (Moawad et al. 1976, Paton 1976, Sporrang et al. 1981) and vasoactive intestinal polypeptide (VIP) (Helm et al. 1981a) released during nerve stimulation have marked

effects on tubal motor activity, including changes in smooth muscle tension. In order to allow for a more detailed quantitative analysis of the sympathomimetic response of the human Fallopian tube in vitro, the spontaneous motor activity was in the present study abolished through depolarization of the smooth muscle membrane by increasing the potassium concentration in the extracellular medium.

The relaxing effect of noradrenaline on the depolarized tubal smooth muscle was studied under conditions when the contractile response was inhibited by phenoxybenzamine (which also efficiently blocks the extra-neuronal uptake of the transmitter) and the neuronal uptake of the amine was blocked by cocaine (cf. Furchgott 1972). The log dose-response curve for the response to noradrenaline showed a parallel shift towards higher agonist concentrations in the presence of increasing concentrations of propranolol, confirming that the relaxation is mediated by beta-adrenergic receptors. Under the standardized conditions used (cf. Furchgott 1972) this shift made it possible to calculate the dissociation constant for the complex between the beta-receptor and propranolol (the K_B value) in different types of tubal preparations at various cyclic stages. The K_B values provide a powerful means to classify the receptor pharmacologically, since the value should be the same for a given receptor irrespective of the type of preparation studied (Furchgott 1972). The K_B value for propranolol i.e. the affinity of this antagonist for the beta-receptor in the tubal smooth musculature, resembled the value for the beta-receptors mediating relaxation of the follicular smooth muscle in the human ovary (Wallis et al. 1977). There was no significant regional or cyclic variation in the affinity of propranolol for the tubal beta-receptor.

The sympathomimetic contraction was studied under similar stand-

ardized conditions as the relaxation using phenylephrine as agonist. Several of the preparations, especially from the circular smooth muscle, did not contract at all. This may be related to the particularly high proportion of beta-receptor activity in this type of smooth musculature (Lindblom et al. 1979). The responding preparations showed a quite varying degree of maximum contraction, and there was a tendency to reduced sensitivity during the ovulation phase. Phentolamine inhibited the contractile response in a competitive manner, confirming the alpha-receptor nature of the response.

The affinity of phentolamine for the alpha-receptor showed a significant difference in the circular smooth musculature, related to low versus moderate-high plasma levels of sex steroids. Thus, the affinity was higher during the follicular phase than during the remainder of the menstrual cycle. No such difference was found for the longitudinal smooth muscle of the isthmus, whereas in the ampulla, the affinity was lower in the follicular phase. It is now well established that alpha-receptors are also present prejunctionally in the sympathetic neuroeffector apparatus (Starke et al. 1977, Langer 1980). They have been designated alpha₂-receptors, in contrast to the traditional postjunctional receptors, which are named alpha₁-receptors. However, recent evidence suggest that the distinction "pre- or postsynaptic" is an oversimplification, and that both alpha₁- and alpha₂-adrenoceptors may, in fact, be present postjunctionally (Lefkoqitz and Hoffman 1980). The affinity of phentolamine for the postjunctional alpha-receptor has been found to be higher ($pA_2 = 7.48$) than for the prejunctional receptor ($pA_2 = 6.18$) (Starke et al. 1975). It is notable that these two pA_2 values correspond well in magnitude to the two populations of

K_B values obtained for the sympathomimetic contractile response of the Fallopian tube at varying stages of the menstrual cycle (approximately 6×10^{-8} and 2×10^{-7} , respectively). It is thus conceivable that the two groups of K_B values reflect two subtypes of alpha-receptors, which may even be identical with the alpha₁- and alpha₂-receptors, each predominating during different parts of the menstrual cycle.

The relationship of the two types of postjunctional alpha-receptors to steroid levels is difficult to evaluate. The present radioimmunoassays of plasma estradiol and progesterone have been used as a tool to define different stages in the menstrual cycle. It has been a general tendency when studying the effects of estradiol and progesterone on various female reproductive tissues to relate the results obtained to the concentration of sex hormones in the blood. It is, however, more reasonable to assume that the biological activity will be governed rather by the concentration in the tissue or the steroid receptors. Although there is a similarity in the general pattern of the cyclic fluctuations in mean steroid concentrations between tubal tissue and plasma, the correlation between these values in each individual patient has turned out to be poor (Batra et al. 1980). This indicates that a more relevant parameter would be the ratio between tissue and plasma concentrations of the steroids which, particularly when the plasma levels are high, minimizes the error of non-specific steroid accumulation in the tissue. Radioimmunological determinations have shown that there is a much higher steroid level in the tubal tissue than in the plasma (Batra et al. 1980), which confirms the presence of estrogen and progesterone receptors in the organ (Robertson et al. 1975, Muechler et al. 1976, Verhage et al.

1980). This indicates that the Fallopian tube, indeed, can be considered as a target organ for ovarian steroids. The present results suggest that the properties of adrenoceptors in different types of smooth musculature in different regions of the tube may be altered under the influence of sex steroids in a way that is not simply reflected in their plasma concentration.

There is ample evidence from studies on laboratory animals that the tubal motor activity is influenced by sex steroids. These effects are probably partly manifested by a direct action on the smooth muscle function (cf. Batra 1980), but also via autonomic neural mechanisms. The adrenergic contribution to these mechanisms has been particularly well studied, and much information is available on the human Fallopian tube: The function of its adrenergic nerves, expressed in terms of their transmitter content, show cyclic fluctuations (Owman et al. 1976, Moawad et al. 1977, Helm et al. 1981c). The sympathomimetic response is changed during the menstrual cycle, conceivably reflecting an altered balance between alpha- and beta-adrenoceptor function (Moawad et al. 1976, Owman et al. 1976, Helm et al. 1981d). The present observations have added to the complexity by suggesting that also the properties of the adrenoceptors, particularly the alpha-receptors, may be modified during various stages of the menstrual cycle.

Supported by the Swedish Medical Research Council (grant No. 14X-5680), Maggie Stephens' Foundation, and from Förenade Liv Mutual Group Life Insurance Company, Stockholm.

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Female sex steroid concentrations in the ampullary and isthmic regions of the human fallopian tube and their relationship to plasma concentrations during the menstrual cycle

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The concentrations of estradiol-17 β (E_2) and progesterone (P) were measured in the ampullary and isthmic portions of the fallopian tube of nonpregnant menstruating women and the cyclic fluctuations were related to the concentrations of these hormones in plasma. The steroid concentrations were determined by radioimmunoassays. There was no significant difference in the isthmic and ampullary concentrations of either steroid in any of the menstrual phases. The mean value for E_2 was highest in the ovulatory phase and for P during the luteal phase. The tissue (per gm)/plasma (per ml) ratio for the steroid concentrations was above unity in all measurements. The ratio for E_2 was highest (isthmus:12, ampulla:8) in the follicular phase and for P (isthmus:26, ampulla:18) during ovulation. Since these highest ratios were attained when plasma steroid concentrations were relatively low they were interpreted as reflections of a maximal receptor contribution. (AM. J. OBSTET. GYNECOL. 136:986, 1980.)

GENERALLY, it is not known how changes in plasma estrogen and progesterone levels during the menstrual cycle are reflected in their target organs. Recent data on the concentration of these hormones in the endometrium and myometrium indicate that there is an overall poor correlation between plasma and tissue levels.¹⁻³ There are as yet no data available on the concentrations of these hormones in the fallopian tube of the human or experimental animals. Such data are important not only from the point of view of steroid dynamics in this organ, but also with regard to the fact

that estrogen and progesterone govern the motor activity of the tube and thereby probably also govern the transport of the ovum toward the uterus.⁴ Steroid-sensitive adrenergic nerves and adrenoceptors in the tube are among the mechanisms which mediate the pattern of tubal motility during the menstrual cycle.⁵ In the present study the concentrations of estrogen and progesterone were determined in the ampullary and isthmic regions of the tube during the follicular, ovulatory, and luteal phases of the cycle of menstruating women and were correlated with the steroid concentrations in plasma.

Material and methods

Material. The fallopian tubes from one or both sides were obtained from 33 menstruating women (31 to 53 years old) subjected to abdominal hysterectomy because of adenomyosis or myoma, or salpingo-oophorectomy for benign ovarian cysts. Material from patients receiving hormone treatment in one form or another was not included.

Morphine chloride (10 mg) and scopolamine bro-

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Supported by the Ford Foundation and the Swedish Medical Research Council (Project No. 4781).

Received for publication March 29, 1979.

Revised June 12, 1979.

Accepted June 19, 1979.

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mide (0.4 mg) were given as preanesthetic medication. Anesthesia was induced by pentothal sodium administered intravenously, followed by inhalation of a mixture of dinitrous oxide and oxygen together with pethidine chloride intravenously. Succinylcholine iodide was given initially as muscle relaxant, followed by pancuronium bromide.

Immediately after removal of the tubes, surrounding connective tissue was dissected away from the muscle wall. A thin (2 mm outside diameter) probe was introduced into the lumen from the ampullary end in order to localize the ampullary-isthmic junction, where the oviduct was divided. A few millimeters of the adjacent isthmic and ampullary portions, as well as the infundibular part of the tube, were discarded. The remainder was lightly blotted on filter paper, weighed, and stored frozen (-18°C) until analyzed.

Venous blood (approximately 10 ml) was drawn from each woman during the operation into heparinized tubes and centrifuged. The plasma was stored frozen at -18°C .

Tissue digestion and extraction of steroids. The frozen tissues were thawed and then digested in a 0.5 ml mixture containing 5% sodium dodecyl sulfate (SDS) and 0.5N NaOH as described previously.⁶ The digested material was extracted three times with 3 volumes of ethyl acetate. The combined extracts were evaporated to dryness under air at $+40^{\circ}\text{C}$. The dried extract, however, contained a significant amount of SDS, which was removed before radioimmunoassay (RIA) by Sephadex LH-20 chromatography, and 6 ml eluate was collected.^{6,7}

Radioimmunoassay. After evaporation of the collected eluate, the residue was dissolved in 1 ml ethyl acetate and suitable amounts, depending on the predicted concentration, of 17β -estradiol (E_2) and progesterone (P) were taken for the respective RIA.

The procedure for RIA of E_2 was that described by Lindberg and associates.⁸ The reliability of this assay for determination of E_2 in tissue extracts has previously been checked.⁷ The recovery of authentic unlabeled E_2 added to digested samples and calculated at the end of the completed procedure was 89.8 ± 4.2 (mean $\pm$ SEM). The intra-assay coefficient of variation in replicate analysis of E_2 was about 10%. Assays were done in a single batch.

The method for P determination was essentially similar to that described by Youssefnejadian and colleagues.⁹ The antiserum (FO 22.5.73), which was a gift from Dr. Kjell Martinsson (Royal College of Veterinary Medicine, Stockholm) and found to be highly specific for P, was used in a dilution of 1/1,500 (vol/vol). Other details have been described previously.⁶ There was an

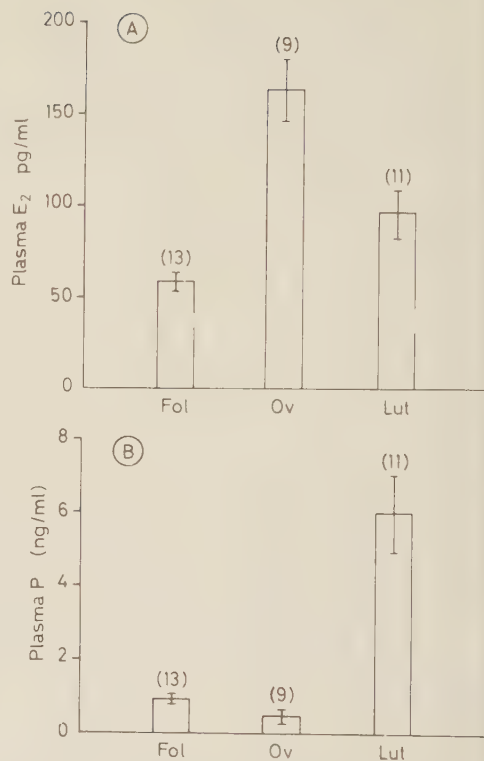


Fig. 1. Plasma concentration of *A*, estradiol- 17β and *B*, progesterone during the follicular (*Fol*), ovulatory (*Ov*), and luteal (*Lut*) phases. Mean values $\pm$ SEM, number of determinations within parenthesis.

almost complete recovery of not only radiolabeled P, but also of unlabeled P (96.5 ± 4.9) added to digested tissue and determined by RIA after extraction.⁶

Protein concentration in the digested tissue was determined in the tissue by Lowry and associates¹⁰ using bovine serum albumin, dissolved in digestion mixture as standard.

Also, E_2 and P concentrations in plasma were determined by these radioimmunoassays.

Definition of cycle stage. The material was divided into three groups with regard to the phase of the menstrual cycle on the basis of plasma steroid levels. The follicular phase included women with plasma $\text{P} \leq 2$ ng/ml and $\text{E}_2 \leq 100$ pg/ml, the ovulatory phase, women with the same plasma P levels, but $\text{E}_2 > 100$ pg/ml, and the luteal phase, those having $\text{P} > 2$ ng/ml. The mean values for the plasma steroid levels in the three groups are illustrated in Fig. 1. Whenever mate-

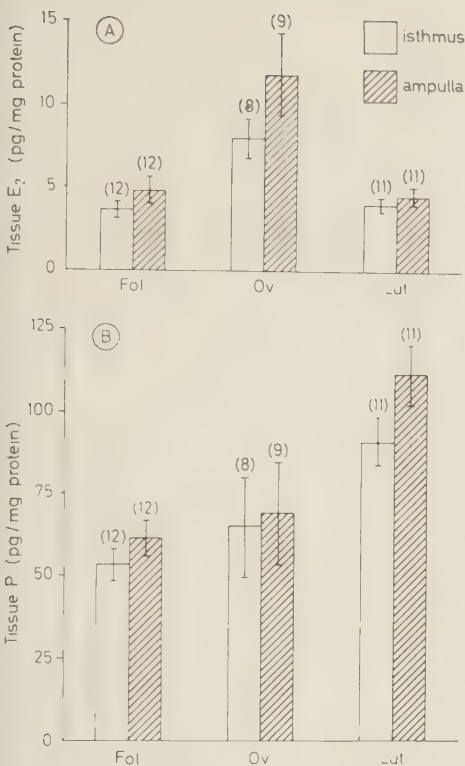


Fig. 2. Tissue concentrations of *A*, estradiol-17 β and *B*, progesterone during the follicular (Fol), ovulatory (Ov), and luteal (Lut) phases. Mean values $\pm$ SEM, number of determinations within parenthesis.

rial was available the cyclic stage was confirmed on hematoxylin-eosin-stained endometrial biopsies. The mean ages of the women in the groups thus defined showed no statistically significant differences from one another.

Chemicals. SDS was purchased from Sigma Chemical Co. E_2 and P were purchased from Ikapharm, Israel. Radioactive [2,4,6,7- H^3] estradiol (102 Ci/mmol) and [1,2,6,7- H^3] progesterone (105 Ci/mmol) were obtained from New England Nuclear Corporation, and Sephadex LH-20 from Pharmacia Fine Chemicals, Sweden.

Statistics. Mean values for the steroid and protein concentrations in the various groups were compared using the unpaired Student *t* test, and the levels in the ampulla and isthmus and left and right tubes, respectively, compared in the paired *t* test. Linear regression analysis was done with the least square method.

Table I. Protein content per gram of tissue in the human fallopian tube at different phases of the menstrual cycle

Phase	Protein (mg/gm wet weight)	
	Isthmus	Ampulla
Follicular	188.4 $\pm$ 16.9 (13)	95.3 $\pm$ 5.3 (13)
Ovulatory	143.1 $\pm$ 12.8 (8)	91.7 $\pm$ 7.2 (9)
Luteal	167.5 $\pm$ 12.8 (11)	97.0 $\pm$ 8.0 (11)

Mean values $\pm$ SEM; number of determinations in parenthesis.

Table II. E_2 and P concentrations (pg/mg protein) in right and left fallopian tubes from 5 women

Isthmus E_2		Ampulla E_2		Isthmus P		Ampulla P	
Right	Left	Right	Left	Right	Left	Right	Left
3.9	2.5	1.6	1.1	39	38	40	38
2.5	1.9	1.9	2.1	54	50	54	54
4.6	3.7	2.7	3.0	61	43	43	55
8.1	8.0	10.1	12.5	68	64	63	74
3.0	2.0	3.0	3.0	48	37	38	38

Paired *t* test showed no statistically significant difference between the concentrations in the right and left tube.

Results

There was a marked difference in the protein content per unit of weight in the isthmus compared to the ampullary region of the fallopian tube (Table I), although the difference between the various cyclic stages was not statistically significant. The values for the concentration of E_2 and P are therefore expressed on the basis of tissue protein. There was, however, in both regions an overall good correlation between steroid concentrations expressed on weight basis and on the basis of tissue protein content ($r = 0.76$ to 0.96 for the two regions during the various phases). The steroid concentrations (amount per protein content) in the two regions are shown in Fig. 2. There was no significant difference in the concentrations between the ampulla and isthmus (paired *t* test) at any of the menstrual phases. The mean values for the tissue concentration of E_2 were highest both in the ampulla and the isthmus around the time of ovulation. On the other hand, P showed a slight insignificant rise from the follicular to the ovulation period, whereas in the luteal phase a pronounced and significant increase was found (Fig. 2). There was a high degree of linear correlation between the ampulla and isthmus with regard to the concentration of E_2 and P ($r = 0.89$ and 0.97 , respectively) during the ovulation phase in contrast to the follicular ($r = 0.43$ and 0.54) and luteal ($r = 0.25$ and 0.04) phases. In five cases the steroid concentration was determined separately in the two tubes from individual

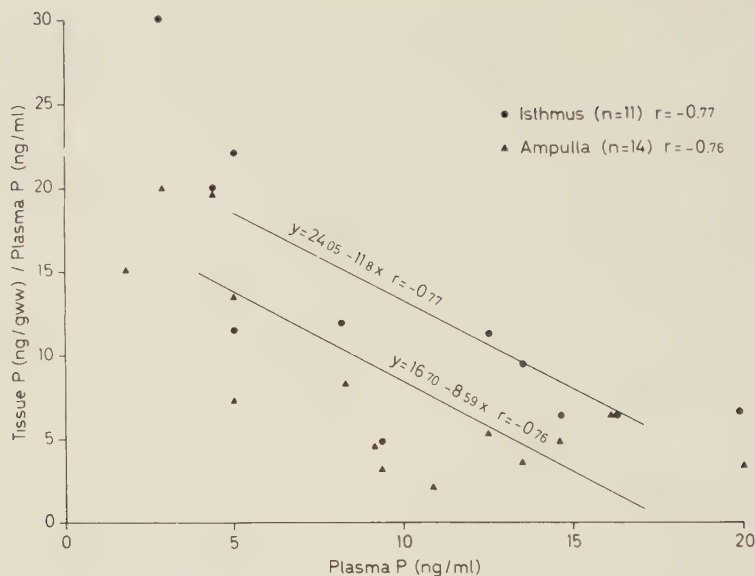


Fig. 3. Correlation between plasma progesterone and tissue/plasma progesterone ratio in follicular phase for isthmus (●) and ampulla (▲) in the linear regression analysis. The equations for the lines and the correlation coefficient (r) are given. The number of women from which the material was obtained is given in parenthesis.

Table III. Ratios between the tissue and plasma concentrations of E_2 and P in the fallopian tube (mean $\pm$ SEM) and the correlation coefficient (r) in the linear regression analysis of the tissue versus plasma concentrations of the steroids

Phase	N	Isthmus		Ampulla		Isthmus		Ampulla	
		E_2 ratio	r	E_2 ratio	r	P ratio	r	P ratio	r
Follicular	12	12.6 ± 2.0	0.03	7.8 ± 1.1	0.57	14.4 ± 2.7	0.50	8.9 ± 1.7	0.71
Ovulatory	8	6.7 ± 1.2	0.40	6.2 ± 1.0	0.39	25.7 ± 4.6	0.91	17.9 ± 4.0	0.81
Luteal	11	8.2 ± 1.4	0.21	4.8 ± 0.7	0.31	3.4 ± 0.5	0.53	2.3 ± 0.4	0.16

N = number of tissues analyzed.

patients (Table II). No significant side-to-side difference was found irrespective of cyclic stage.

The ratios between tissue and plasma steroids in the two regions at the various menstrual stages are presented in Table III. It can be seen for both the isthmus and ampulla that, whereas the capacity to accumulate E_2 was most prominent during the follicular phase, it was highest in the ovulation phase for P. As also shown in Table III, this agrees with a high degree of linear correlation between the plasma and tissue values for P during the ovulation phase.

It was found that there was a tendency for an inverse relationship between the tissue/plasma ratio for P and

the plasma level of the hormone in the individual patients. Assuming the tissue/plasma ratio of P reflected the amount of available P receptors, this ratio was plotted against the plasma P concentration in order to test the possibility of receptor inactivation. Linear regression analysis showed a fairly good correlation between the two parameters for both isthmus and ampulla during the follicular phase. This analysis was not performed for the ovulation phase because of the interference by the high E_2 level, and it was not considered feasible during the luteal period when the levels of P are too high for detection of any variability in P receptors (Fig. 3).

Comment

The tissue concentrations of E_2 and P were measured in the ampullary and isthmus regions of the human oviduct during the follicular, ovulatory, and luteal phases of the menstrual cycle as defined on the basis of plasma steroid levels and confirmed by histologic examination of the endometrium in cases where the operation also included hysterectomy. There are no previous data available on the content of E_2 or P concentration in the human tube. The concentration of either steroid in the tube reported here was largely similar to that reported for the human nonpregnant myometrium by Runnenbaum and colleagues.³

However, the P value for tubal tissue during the proliferative phase obtained in this study was about ten times higher than the corresponding myometrial value reported by Runnenbaum and associates,³ but in the same order of magnitude as that recently found by us (to be published). The discrepancy may be due to an inadequate extraction of P from the myometrial tissue.⁶ The cyclic fluctuations of the hormones in the tubal tissue followed the same pattern as that reported for the myometrium presented by Runnenbaum and associates³ in the myometrium. Our results show that in general the correlation between the tissue and plasma levels is poor, and that there was always a considerably higher steroid level in the tissue than in plasma, although the tissue/plasma ratio for the steroid concentrations varied during various phases. This suggests that the fallopian tube can be considered as a target organ for female sex steroids. Indeed, the existence of estrogen and progesterone receptors has recently been demonstrated in the fallopian tube.^{11, 12}

A comparison of the hormone concentrations in the isthmus and ampulla is best made on the basis of protein content, since this was different per unit of wet weight in the two regions. The protein concentration was considerably higher in the isthmus which is not surprising in view of the fact that this is a more muscular tissue compared to the ampulla. In fact, the protein content in the isthmus compares well with that recently found for the myometrium.¹³ However, there was no

significant difference in the protein concentration of either the ampulla or the isthmus in the various cyclic stages, which justifies the comparison of the steroid levels between different phases in the same tissue. According to this there was no statistically significant difference in either the E_2 or P level of the ampulla and isthmus in any of the phases, although the mean values for the ampulla were always slightly higher than for the isthmus.

The changes in the hormonal concentration in both tissues from one phase to another are best compared in relation to plasma levels, since the concentration in the blood is also changing during the menstrual cycle. We have therefore calculated the tissue/plasma ratio for such a comparison. This ratio was highest for both isthmus and ampulla in the follicular phase for E_2 and in the ovulatory phase for P.

The tissue/plasma ratio of the steroid concentrations can be considered to reflect the steroid accumulating ability of the tissue, which should be influenced to a large degree by the concentration of the receptors for each hormone. This should particularly be the case when the plasma concentrations are relatively low. When plasma hormone concentration is high so would be the concentration in the tissues, but the contribution from binding of hormone to nonspecific sites (not receptors) in the tissue might be considerable under these conditions. Accordingly, in our data the receptor ability would be best expressed during the follicular phase for E_2 and during ovulation for P. In the luteal phase, however, besides the changes in the plasma concentration that would influence the tissue concentration, there might be additional effects of P on E_2 and P receptors. P has been shown to significantly reduce receptors for both E_2 and P in the uterus.¹⁴⁻¹⁶

The general conclusion from the present data is that although there was a similarity in the patterns of the cyclic fluctuations for the mean steroid concentrations in the tube and plasma, the correlation between the individual plasma and tissue values, as established in the linear regression analysis, was poor.

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Vasoactive intestinal polypeptide nerves in the human female genital tract

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VIP, a recently recognized neuropeptide, has been demonstrated by immunohistochemistry in nerves of the human female genital organs. Such nerves were most numerous in the isthmus part of the fallopian tube and in the cervix. They were found in smooth muscles, around blood vessels, and beneath the epithelium. Immunoreactive VIP nerve cell bodies were found in paracervical tissue, suggesting a local origin of genital VIP nerves. Besides the well-known adrenergic and cholinergic nerves, peptidergic nerves may constitute a new component in the autonomic nervous system. (AM. J. OBSTET. GYNECOL. 136:349, 1980.)

VASOACTIVE intestinal polypeptide (VIP) is one of a number of recently recognized neuropeptides with ubiquitous occurrence in the body. Others are substance P, somatostatin, and enkephalin. They have all been demonstrated in the brain as well as in nerves of peripheral organs.¹ In the periphery the peptide-containing nerves probably belong to the autonomic nervous system although they seem to be distinct from adrenergic and cholinergic nerves. The peptidergic nerves form a prominent part of the intramural neuron system in the gut. From the fact that the recognized neuropeptides have a number of biologic actions it is tempting to assume that they function as transmitters. Admittedly, the evidence for this is still incomplete.

VIP nerves occur in the male and the female genito-

urinary tract of several mammals.^{2, 3} In the present report we describe the regional distribution of VIP nerves in the human female genital tract, information which has previously not been accessible.

Material and methods

Tissue specimens from various parts of the genital tract were obtained from 20 women undergoing hysterolapingo-oophorectomy because of adenomyosis, menometrorrhagia, and/or myoma. Material from seven human fetuses was obtained at legal abortions performed by hysterotomy at 18 to 23 weeks' pregnancy. Immediately after the organs had been excised small tissue pieces were taken from the ovaries, the ampulla, and isthmus of the fallopian tube and the fundus, corpus, and cervix of the uterus. The tissue pieces were frozen in a mixture of propane and propylene at the temperature of liquid nitrogen and freeze dried. They were then exposed to diethylpyrocarbonate vapor for 3 hours at 55° C⁴ and embedded in paraffin in vacuo. Sections were cut at 5 µm, deparaffinized, and processed for immunofluorescence⁵ or immunoperoxidase staining,⁶ with a previously characterized anti-VIP serum (No. 5603; generously supplied by Drs. J. Fahrenkrug and O. Schaffalitzky de Muckadell, Bispebjerg Hospital, Copenhagen, Denmark). At least 30 sections from each specimen (two to

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Supported by grants from the Ford Foundation (680-0383A) and the Swedish Medical Research Council (04X-4499).

Received for publication January 2, 1979.

Revised March 9, 1979

Accepted March 16, 1979.

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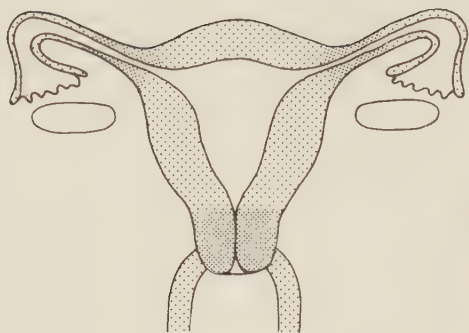


Fig. 1. Schematic outline of distribution and relative frequency of VIP-containing nerve fibers in human female genital tract. Density of stippling relates to the frequency of nerve fibers.

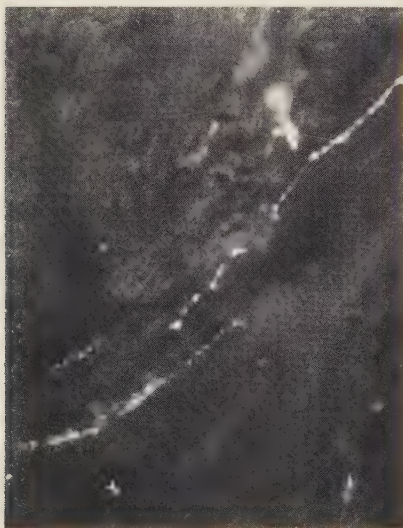


Fig. 2. Immunofluorescent VIP nerve terminals running along smooth muscle bundles in the cervix. ($\times 300$.)

three from each location and woman) were examined microscopically. Immunofluorescence was examined in a Leitz epiillumination fluorescence microscope with standard filter setting No. 3. Immunoperoxidase staining was examined in a standard light microscope. The frequency of nerves was evaluated subjectively. For controls in the immunohistochemical procedure we used antiserum inactivated by the addition of excess



Fig. 3. Immunofluorescent VIP nerve terminals (arrows) immediately beneath the vaginal epithelium (*ep*). ($\times 300$.)



Fig. 4. Clusters of nerve cell bodies at the uterovaginal junction, some of which display a weak to moderate VIP immunoreactivity. (Immunoperoxidase staining; $\times 300$.)

antigen (30 nmol of pure porcine VIP per milliliter of diluted antiserum). Staining was regarded as specific only if it failed to appear in the controls. For simplicity, VIP immunoreactivity is referred to as VIP.

Results

For a schematic survey of the regional distribution of VIP nerves in the female genital tract, see Fig. 1.

In the endocervix there was a moderate to rich number of VIP nerves, mostly terminals, but more coarse nerve trunks were also found. They were seen to surround small blood vessels and to run along the bundles of smooth muscles cells (Fig. 2), sometimes forming a fiber network. In addition, some terminals were seen parallel to the cervical glands ending beneath the surface epithelium. The density of innervation appeared greater in the endocervix than in the exocervix and vaginal fornices, where scattered terminals were found in the smooth muscle layer and in a subepithelial position (Fig. 3). In the isthmus region of the cervix a small to moderate number of VIP nerves was found in the smooth muscle close to the endometrium; deeper in the myometrium there was a gradual decrease in the nerve supply. In the corpus part of the uterus only few scattered VIP nerves were observed in the smooth muscle.

No VIP nerves were found in the ovary. In the fallopian tube a moderate to large number of VIP terminals were observed in the isthmus part, predominantly localized beneath the epithelium. The nerve supply was somewhat greater in the lamina propria of the mucosa than in the smooth muscle layer. In the other parts of the tube only few scattered VIP terminals were observed in the smooth muscle.

In the fetuses immunoreactive VIP was localized exclusively in nerve cell bodies occurring in clusters in the paracervical tissue. The cell bodies displayed weak to moderate VIP immunoreactivity (Fig. 4).

Comment

The peripheral autonomic nervous system classically consists of adrenergic and cholinergic nerves. Recent years have witnessed the demonstration of peripheral (and central) nerves, with characteristic distribution and properties and containing polypeptides (substance P, VIP, somatostatin, or enkephalin) as presumed neurotransmitters. The distribution of VIP and substance P nerves have been described in the genitourinary tract of various species^{2, 3} but until now not in human subjects. VIP is known to have powerful biologic actions, among them smooth muscle relaxant and vasodilatory effects.

The intramural distribution of VIP nerves in the smooth muscle, around small blood vessels, and beneath epithelia in the human female genital tract suggests that the VIP nerves may participate in the regulation of smooth muscle activity, local blood flow, and epithelial functions.

The presence of VIP immunoreactive nerve cell bodies in the paracervical ganglia suggests that uterovaginal VIP nerves may have a local origin. This view is supported by denervation experiments in the cat which have shown that the VIP nerves in the female genital tract originate from the paracervical ganglia.⁷ The absence of VIP nerves in the fetal target organs suggests a fairly slow development of the VIP nerve system.

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Vasoactive Intestinal Polypeptide (VIP) in the Human Female Reproductive
Tract: Distribution and Motor Effects

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ABSTRACT

The distribution and concentration of vasoactive intestinal polypeptide (VIP) in the human female genital tract were studied by means of immunohistochemistry and immunochemistry. Further, the effect of VIP (10^{-10} - 10^{-7} M) on the mechanical activity of uterine smooth muscle was investigated. Generally, VIP immunoreactive nerve fibers were distributed among smooth muscle cells and around vessels. They were most abundant in the isthmic part of the Fallopian tube, the outer and inner ostium of the cervix, and in the vagina. The tissue concentration of VIP measured by immunochemistry was in good agreement with the immunohistochemical findings. VIP inhibited the spontaneous smooth muscle activity in strips from the Fallopian tube and the cervical region, but had no effect on strips from the uterine corpus. The data support the view that VIP may play a physiological role in the local control of smooth muscle motility in the human female reproductive tract.

INTRODUCTION

Vasoactive intestinal polypeptide (VIP) — a 28 amino acid peptide structurally related to secretin, pancreatic glucagon and gastric inhibitory peptide (GIP) — was isolated from the porcine small intestine by Said and Mutt (1970). Recently, VIP has been shown to have a widespread distribution in the body localized in neurons, and evidence is accumulating that the peptide represents a neurotransmitter (e.g. Fahrenkrug, 1979). VIP has been demonstrated in the genital organs of various species (Alm et al., 1977, 1980 a and b; Larsson et al., 1977a and b; Goodnough et al., 1979; Ottesen et al., 1979a. In laboratory animals the peptide shows a dose-dependent inhibitory effect on the myoelectrical activity and on the contractile response of the myometrium stimulated with oxytocin or prostaglandin $F_{2\alpha}$ (Ottesen et al., 1979a and b, 1980), and it increases myometrial blood flow (Carter et al., 1980). In the present study we have examined the occurrence and distribution of the VIP-containing nerves throughout the human female reproductive tract by immunohistochemistry and quantified the content in tissue extracts by radioimmunoassay. In addition we have studied the effect of VIP on the spontaneous cervical, myometrial, and tubal smooth muscle activity.

MATERIALS AND METHODS

Material

Tissue specimens were obtained from 53 women undergoing gynecological surgery: 31 hysterectomies (fibromyoma, adenomyosis; age 27–54 years), 14 salpingectomies (sterilisation; age 31–43 years), 3 salpingoophorectomies (benign ovarian cysts; age 22–65 years), and 5 vaginal operations (descensus uteri; age 21–70 years). Only tissue with normal gross appearance was used. Women receiving hormones or β -receptor antagonist drugs were not included. Prior to the vaginal operation, the older patients (aged 50–70 years) were treated with estradiol. Preanesthetic medication included atropine (0.5 mg) and meperidine (50 mg). Anesthesia was induced by Pentothal administered intravenously, followed by inhalation of dinitrous oxide and oxygen together with halotane in a semi-closed system.

Immunohistochemistry

After removal during the operations, specimens were taken from the ovary, ampulla and isthmus of the Fallopian tube, the myometrium, the endometrium, the cervical mucosa, the external and internal ostium of the cervix, and the vagina from 20 of the women. Some specimens were immediately frozen in a mixture of propane and propylene cooled by liquid nitrogen. After freeze-drying, the preparations were fixed with diethyl pyrocarbonate vapour and embedded in paraffin. Other specimens were immersed in ice-cold fixation solution (4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.1) for 24-48 hrs. They were then rinsed repeatedly in cold buffer containing 5% sucrose for 43 hrs, frozen on dry ice and subsequently sectioned in a cryostat. Sections from paraffin-embedded material and cryostat sections were subjected to the indirect immunofluorescence method of Coons et al. (1955) or the peroxidase-antiperoxidase (PAP) technique of Sternberger (1974) for demonstration of VIP.

The VIP antiserum (No. 5603), which has been characterized in detail elsewhere (Larsson, 1976; Fahrenkrug, 1977, 1978), was used in dilution 1:80 (for the immune fluorescence) or 1:5,600 (for the PAP procedure). The site of antigen-antibody reaction was revealed by fluorescein isothiocyanate labelled goat anti-rabbit IgG (Statens Bakteriologiska Laboratorium, Stockholm, Sweden), diluted with 1:20 or by PAP (Capel Labs, Downington, Penn., USA), diluted 1:320. Controls included sections incubated with VIP antiserum inactivated by addition of highly purified porcine VIP (10 nmol/ml diluted antiserum).

Immunochemistry

Specimens were taken from the areas listed in Table 1. They were immediately frozen on dry ice and weighed. The frozen tissue was stored at -20°C until extraction. VIP was extracted from each tissue specimen by homogenization at 0°C in a glass homogenizer containing 5 vol. of acidified ethanol (70% ethanol containing 0.74% hydrochloric acid w/v). After centrifugation (1,500 xg) at 4°C for 15 min the supernatant was decanted and the residues resuspended and homogenized with additional 5 vol. of acidified ethanol. After centrifugation the combined supernatant fractions were evaporated to dryness in vacuo. The samples were reconstituted and diluted in assay buffer (0.04 M sodium phosphate buffer, pH 7.4, containing 58 M human serum albumin and 0.1 M sodium chloride), and measured in at least three dilutions using antiserum No. 5603-6 (Fahrenkrug, 1977, 1978).

In vitro tension recordings

Material from the uterus and the Fallopian tube were used. The serosa was removed from the tubes which were cut at the ampullary-isthmic junction, and from each part a 4 mm long cylinder was prepared for registration of circular activity and a 6 mm long piece was used for longitudinal recordings.

Specimens (2 x 2 x 10 mm-size) from the uterine corpus were dissected longitudinally from non-pathologic areas. The cervical muscle strips (2 x 2 x 10 mm size) were cut either longitudinally or circularly. The specimens were mounted for isometric recordings with Harvard or Grass FT03C force-displacement transducers and suspended in 10 ml or 40 ml organ baths kept at 37°C containing Krebs solution bubbled with a mixture of 5% CO₂ in O₂. The buffer solution had the following composition (concentration in mmoles per litre): NaCl 118, KCl 4.5, CaCl₂ 2.5, MgSO₄ 1.0, KH₂PO₄ 1.0, NaHCO₃ 25, and glucose 6.0; pH = 7.4 (range: ± 0.15). The isometric muscle activity was recorded by means of a Watanabe WTR 331 Linearrecorder Mark III or Grass model 7B polygraph. Spontaneous activity developed within a few min after mounting. The experiment started when the spontaneous activity showed a regular rhythm of contractions. Highly purified porcine VIP was added in a cumulative way resulting in final concentrations of 10⁻¹⁰ - 10⁻⁷ M in the bath. The reduction in motor activity induced by VIP was measured planimetrically as the area under the record curve in relation to the baseline during the 3-min period of maximal effects as compared to the same period of time immediately before addition of the peptide.

RESULTS

Immunohistochemistry

No VIP nerves could be detected in the ovary. The Fallopian tube contained VIP nerves in a low to moderate number distributed in the non-vascular smooth musculature (Fig. 1) as well as beneath the epithelium. They were more frequent in the isthmic part than in the ampulla. Only a few VIP nerves were observed in the uterine corpus; also here they were usually not related to blood vessels (Fig. 2). The relationship of the immunoreactive nerve fibres to the vascular bed could be secured by alternating between fluorescence (dark-field) and phase contrast optics in serial sections. In the cervix, on the other hand, VIP nerves were numerous. They occurred in the connective tissue and among bundles of smooth muscle (Fig. 3),

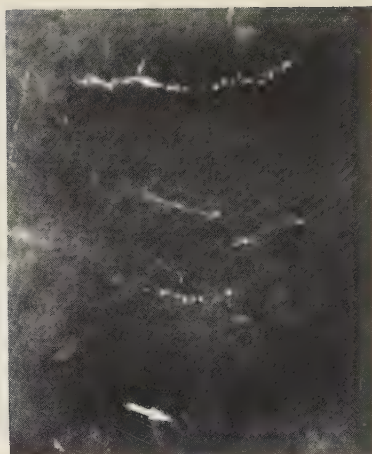


Fig. 1

Tubal isthmus. Nerve fibers displaying VIP immunofluorescence among bundles of smooth muscle, without relation to blood vessels (arrow). X 200.



Fig. 2

Uterine corpus. Few VIP fibers are seen, none of them related to blood vessels. X 200.

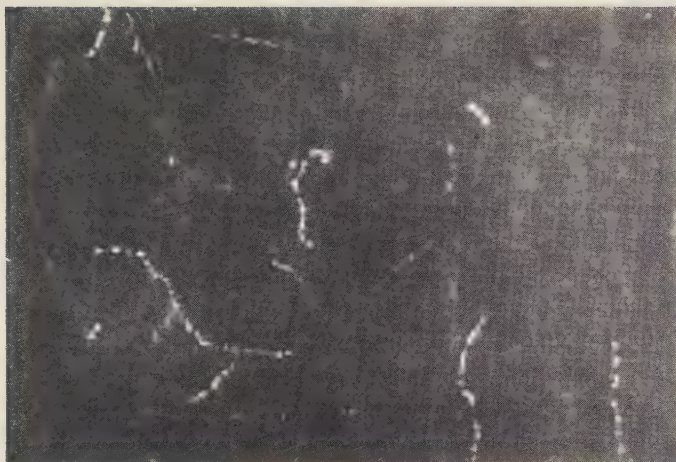


Fig. 3

Uterine cervix. Numerous VIP-containing nerve fibers ramifying among the smooth muscle bundles, without relation to blood vessels (which was confirmed by phase contrast optics in this and adjacent sections). X 200.

as well as around blood vessels (Fig. 4). In the vagina, VIP nerves were seen in fairly large number beneath the epithelium (Fig. 5a), and they were regularly found also around blood vessels (Fig. 5b).

Immunocytochemistry

The concentration of VIP in the different parts of the human female genital tract is summarized in Table 1. The VIP content was significantly higher in the isthmus of the Fallopian tube than in the rest of the tubal tissue. It was significantly higher in the cervix and vagina compared to the remainder of the genital tissue analyzed.

In vitro tension recordings

The motor activity of the spontaneously contracting Fallopian tube is a complex function of smooth muscle tone as well as amplitude and frequency of the individual deflexions. Changes in tubal smooth muscle activity have been defined as changes in the surface area under the curve during a standard period immediately before and after administration of VIP. Thus defined, VIP inhibited in a dose-dependent manner the motor activity of the tubal (Fig. 6) and cervical smooth muscle preparations. Representative tracings from the various types of smooth musculature tested from the tube and cervix are illustrated in Fig. 7. Both the longitudinally and circularly oriented smooth musculature responded with a reduction of motor activity. The minimum effective dose of VIP was in all preparations 10^{-10} - 10^{-9} M. VIP had no effect on the muscle strips from the uterine corpus (Fig. 8).

DISCUSSION

The present study has demonstrated the presence of VIP-containing nerve fibers in the human female reproductive tract by immunohistochemistry as confirmed by immunochemical determination of VIP. The VIP fibers were most abundant in the isthmus of the Fallopian tube, in the wall of the internal and external ostium of the cervix canal, and in the vagina. The concentration of VIP measured in the different regions by radioimmunoassay agreed with the relative number of VIP nerves found by immunohistochemistry. The tissue concentration of the peptide showed a considerable variability in the various parts of the genital tract. In the

TABLE 1. Distribution and concentration of VIP, measured by radioimmunochemistry and expressed as pmol/g wet weight, in the human female reproductive tract.

Specimen	Median	(n)	95% confidence limits	statistics††
Ovary	0.1	(6)	0 - 2.4	
Fallopian tube fimbria	1.0	(5)	0.1 - 1.6	
ampulla	1.9	(27)	0.3 - 2.8	n.s.
isthmus	4.4	(26)	1.4 - 7.2	$p \leq 0.05$
Uterine corpus myometrium	1.6	(30)	0.9 - 2.7	n.s.
Cervix	16.5	(10)	6.4 - 26.6	$p \leq 0.05$
internal ostium	11.4	(8)	0.2 - 25.5	n.s.
external ostium	3.6	(6)	0.2 - 11.5	$p \leq 0.05$
Portiot	23.8	(6)	1.4 - 45.0	n.s.
Vagina	0.5	(18)	0 - 0.8	$p \leq 0.05$
Uterine corpus endometrium	1.7	(9)	0 - 3.0	n.s.
Cervical mucosa				

+ Transverse section of the portio

†† Mann-Whitney rank sum test

n.s. = Non-significant

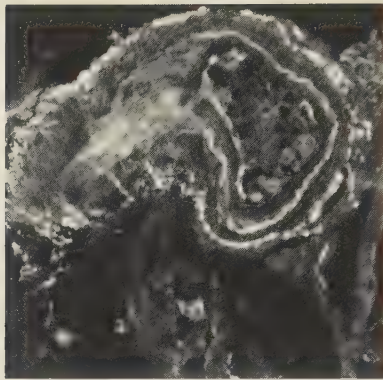


Fig. 4

Uterine cervix. Many of the VIP-containing nerves were associated with blood vessels of varying calibre. The internal elastic membrane of this large vessel, partly transversely and partly longitudinally sectioned, is autofluorescent. The VIP nerves are restricted to the vascular adventitia. X 200.

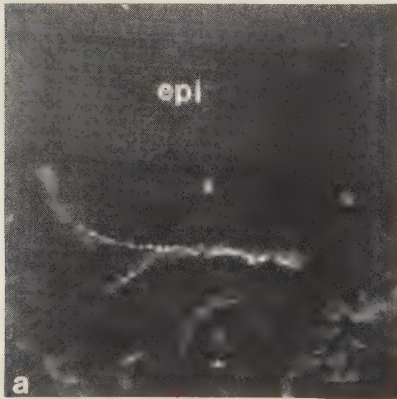


Fig. 5 a

Vagina. Single, branching VIP fiber running beneath the epithelium (epi). Collagen fibers show slight autofluorescence.



Fig. 5 b

Vagina. VIP nerve in the connective tissue branching around small blood vessel (arrow). X 200.

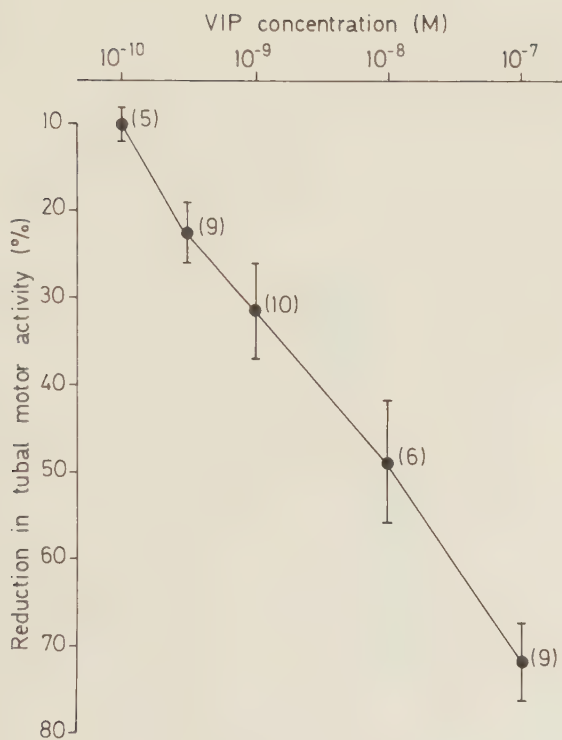


Fig. 6

Reduced contractile activity of the tubal isthmus (circular musculature) when increasing doses of VIP are added in a cumulative manner. The final mean concentrations in the bath are given; vertical bars show the standard errors. Number of observations within parenthesis.

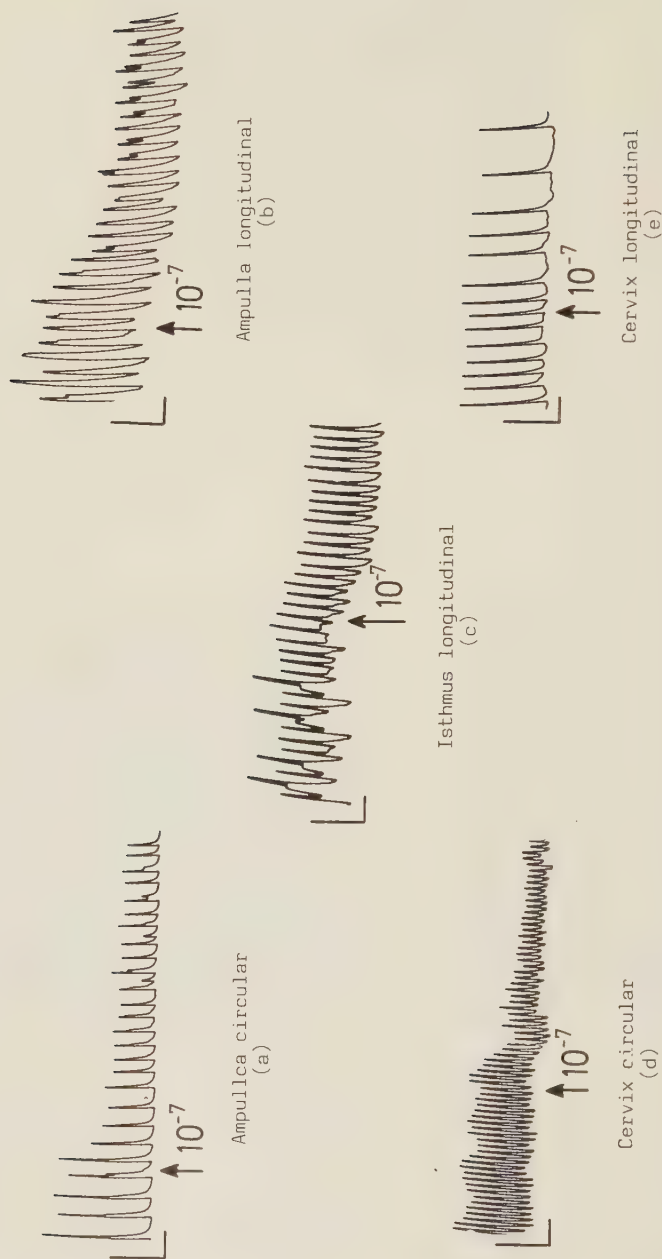


Fig. 7. Representative tracings showed VIP-induced reduction in spontaneous motor activity of the following smooth muscle preparations: a) tubal ampulla, circular; b) tubal ampulla, longitudinal; c) tubal isthmus, longitudinal; d) cervix, circular; and e) cervix, longitudinal. Horizontal calibration: 1 min; vertical calibration: 200 dynes.

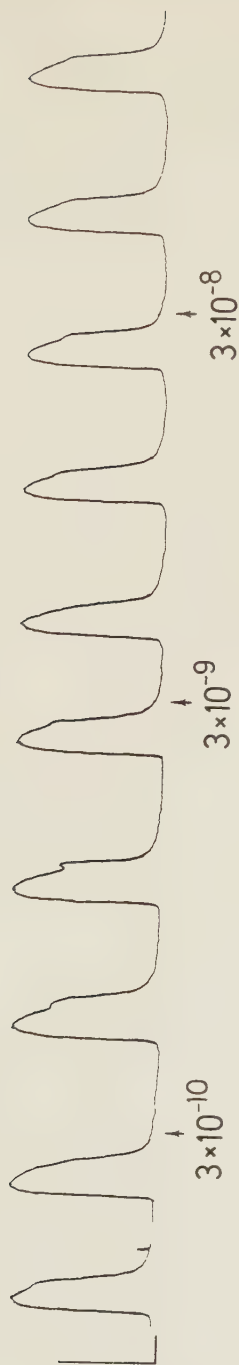


Fig. 8

Representative tracing showing absence of VIP effect on spontaneously contracting myometrial strip. Three concentrations (3×10^{-10} , 3×10^{-9} , 3×10^{-8} M) were tested (arrows). Horizontal calibration: 2 min. Vertical calibration: 5000 dynes.

present material, unselected with regard to phase, this may be due to cyclic variations in the neural content of VIP and/or a reflection of alterations in tissue water content, since values were expressed per unit wet weight. The distribution agreed with that previously reported for other mammalian species through the density of the innervation in humans was lower than e.g. in the cat (Ottesen et al., 1980). There is evidence from immunohistochemical studies in combination with denervation experiments carried out on laboratory animals that the VIP innervation in the reproductive organs originates in local ganglia, present in the utero-vaginal junction (Alm et al., 1980b). These ganglia represent, to a smaller or greater extent depending on animal species, the site of origin also for the well-developed adrenergic innervation to these organs (Owman et al., 1966; Rosengren and Sjöberg, 1967; Thorbert et al., 1977). The *in vitro* tension recordings have shown a dose-dependent inhibitory effect of VIP on the spontaneous smooth muscle contractions, as demonstrated on strips from the Fallopian tube and the cervical region. An inhibition of the mechanical activity of smooth muscle has previously been demonstrated on strips from the genital tract in other mammalian species (Ottesen et al., 1980), and from the gastrointestinal and respiratory tracts (Piper et al., 1970). VIP had no effect on the myometrial smooth muscle strips. This might be related to the low density of VIP-containing nerves in this tissue, where they were present mainly in association with blood vessels. In other mammalian species the ability of VIP to relax myometrial strips from different species is well correlated to the concentration of endogenous VIP measured in the tissue.

The presence of VIP-containing nerves in the human female genital tract, and the dose-dependent inhibitory effect of VIP on its smooth muscle activity, suggests that the peptide may play a role in the local control of the smooth muscle cells. This is supported by the observation that electrically induced relaxation of smooth muscle strips from the tubal isthmus is only partly blocked by adrenergic and cholinergic antagonists (Helm et al., 1981).

The ovary had the lowest concentration of VIP among all tissues analyzed, in agreement with the absent or very scarce VIP innervation shown with immunohistochemistry. The organ is, however, well supplied with adrenergic and cholinergic nerves, innervating blood vessels, the smooth musculature in the follicles, and endocrine cells (Owman et al., 1979). In many respects the autonomic innervation

of the ovary is anatomically and functionally separated from that of the remainder of the reproductive tract. The Fallopian tube, uterus and vagina were heavily innervated by adrenergic nerves which have a complex origin, being partly derived from local ganglia in the utero-vaginal junction (Owman et al., 1966; Rosengren and Sjöberg, 1967; Thorbert et al., 1977). This adrenergic innervation shows a number of functional and neurobiological properties that are different from the rest of the peripheral sympathetic nervous system (see Thorbert, 1978). The cholinergic innervation of these structures is essentially restricted to the extrinsic part of the vascular bed and to the mucosa (Thorbert et al., 1977; Hammarström, 1980). The system of VIP nerves seems to represent another widespread innervation of the female reproductive tract probably involved in the regulation not only of the vascular bed but also of smooth muscle function, particularly within the sphincter regions constituted by the tubal isthmus and uterine cervix (Wallis et al., 1980).

ACKNOWLEDGEMENT

This work was supported by grants from the Danish Hospital Foundation for Medical Research (Region Copenhagen, The Faroe Islands and Greenland: No. 78-13 and 79-16); Thomas Bartholins Fund; Novo's Fund; the Danish Medical Research Council (No. 512-16111); the Faculty of Medicine, University of Lund and the Swedish Medical Research Council (Grant no. 14X-5680). The skilful technical assistance of Susanne Bundgaard, Eva Engelbert, Kristine Fogelström, Barbro Gustavsson, Anita Hansen, Lene Poulsen and Helle Trelde is gratefully acknowledged. Highly purified porcine VIP was kindly donated by Prof. V. Mutt, Karolinska Institutet, Stockholm, Sweden.

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NEUROGENIC RELAXATION MEDIATED BY VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)
IN THE ISTHMUS OF THE HUMAN FALLOPIAN TUBE

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SUMMARY

The smooth musculature of the Fallopian tube is important for normal ovum transport, fertilization and implantation. Little is known about the factors controlling the motor activity of the isthmic sphincter. Studies were performed on smooth muscle preparations from the human tube in vitro. Electrical field stimulation of the nerves in the isthmic region reduced the motor activity, particularly in the circular muscle. The response was unaffected by adrenergic and cholinergic antagonists, but blocked by tetrodotoxin, suggesting a neural involvement. Vasoactive intestinal polypeptide (VIP) was considered a likely candidate for the neural mediation of this response in view of the high density of VIP-containing nerve fibres in this region, and in view of the fact that exogenous VIP causes a marked reduction of the tubal motor activity. To test whether VIP might be the endogenous mediator of this effect, nerve stimulation was carried out in the presence of large amounts of exogenous VIP in order to occupy all VIP receptors; the motor inhibitory action of VIP was counteracted by vasopressin. Under these conditions, nerve stimulation failed to reduce isthmic motor activity. This was not due to vasopressin since reduction occurred in the presence of this peptide alone. The results suggest that VIP is responsible for the neurogenic inhibition of motor activity in the isthmus region of the human Fallopian tube.

Human Fallopian tube; smooth muscle activity; transmural nerve stimulation; non-adrenergic, non-cholinergic relaxation; vasoactive intestinal polypeptide

INTRODUCTION

Several important events in the reproductive process - spermatozoal transport and capacitation, ovum pick-up and transport, and transport and nourishment of the fertilized egg - are known to occur in the Fallopian tube. Despite intensive research during more than half a century, our knowledge about the role of the tube in these functions is incomplete (12).

Following ovulation, the ovum is transported in the oviduct, first to the site of fertilization and then to the uterus for implantation. The ovum transport is not a simple continuous progress: the ova show complex "to-and-fro" movements while they approach the uterus, probably governed by a combination of muscular and ciliary actions (14). In women, the ova are retained in the ampulla for about 3 days, followed by a transit through the isthmus in the course of some 10 hrs. This "tubal locking" appears to be essential for normal fertilization. Premature entry of the fertilized egg into the uterus prevents implantation, and the embryo is expelled. Compounds that accelerate ovum transport through the oviduct may therefore prove useful in the development of new antifertility drugs. There is strong reason to assume that the retention and transit of the ovum during its transport towards the uterus is controlled by local neurogenic mechanisms operating at the isthmus level (3). In immunohistochemical studies it has recently been found (1) that the human Fallopian tube, besides its adrenergic innervation (11), is supplied with numerous nerve fibres containing vasoactive intestinal polypeptide (VIP); their terminals are particularly concentrated in the smooth muscle of the isthmus. The VIP fibres probably originate in pelvic ganglia located adjacent to the uterine cervix (2).

The motor activity of the human tube is inhibited by a non-adrenergic, non-cholinergic nerve mechanism (8), and since exogenous VIP reduces the motor activity (15), it has been suggested that VIP may be the neurotrans-

mitter that mediates the relaxation of the isthmic sphincter (15). The present study provides evidence that nerve-induced motor inhibition in the tube is, indeed, linked with activation of VIP receptors in the smooth musculature.

MATERIALS AND METHODS

Human Fallopian tubes were obtained from 65 adult, regularly menstruating women (aged 38 - 50 years) during abdominal hysterectomy (because of myoma), salpingo-oophorectomy (for benign ovarian cysts), or salpingectomy (for sterilization). Fifty-four tubes in which the circular musculature responded with a distinct reduction in motor activity during the initial electrical stimulation were included in the study. The cyclic stage was estimated on the basis of the patient's menstrual cycle and from preoperative plasma values of estradiol and progesterone determined by radioimmunoassay (6,16).

Immediately after removal the oviducts were kept in ice-cold Krebs-Ringer buffer solution until they were mounted in an organ bath within 1 hr after removal. A thin (2 mm o.d.) probe was introduced into the lumen from the ampullary end in order to localize the ampullary-isthmic junction, where the oviduct was divided. A few mm of the adjacent isthmic portion was discarded. The following 10 mm of the isthmus was used for recording of isometric smooth muscle activity in an organ bath.

A 4 mm long cylinder was cut for registration of circular muscle activity; the remaining 6 mm long piece was used for recording of motor activity in the longitudinal muscle. The pieces prepared for registration of circular muscle activity were mounted between two L-formed metal holders, whereas those for recording of longitudinal muscle activity were stretched between two metal hooks using silk ligatures. Both preparations were connected to Grass FT03C force-displacement transducers, and their signals were amplified and recorded by a Grass model 7B polygraph. Electrical field stimulation was applied by two platinum electrodes placed 10 mm apart on either side of

the preparations. Biphasic pulses (10 V over the electrodes) were used at a frequency of 16 Hz and at a low pulse duration (1 msec) in order to stimulate the nerves while avoiding myogenic effects (10). The organ baths contained a modified Krebs-Ringer solution. The buffer solution had the following composition (concentrations in millimoles): NaCl 118, KCl 4.5, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 2.5, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ 1.0, NaHCO_3 25, KH_2PO_4 1.0, glucose 6.0. The temperature of the baths was thermostatically maintained at 37°C, and the buffer solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 giving a mean pH of 7.40 (range 7.25 - 7.55). All preparations were given an initial passive tension of 500 dynes and were allowed to accommodate for 45-60 min. During this period the mean tension decreased by 150 - 200 dynes and the preparations started to contract spontaneously.

RESULTS AND DISCUSSION

The characteristics of the spontaneous motor activity in the isthmic part of the human Fallopian tube have been described in detail elsewhere (5). The motor response of the preparation to the administration of norepinephrine (5) and to transmural electrical stimulation is either an increase or a decrease in activity, and often the effect is biphasic. The net effect seems to depend on, among other things, the balance between α - and β -adrenoceptor activity in the smooth muscle at different stages of the menstrual cycle (5,9). In the circular smooth muscle of the tube there seems to be a general predominance of β -receptor activity, whereas the α -adrenergic receptor activity appears to predominate over the β -receptor activity in the longitudinal smooth muscle (5,8). The responses in the series of tests carried out in the present study were most clear-cut in the circular isthmic musculature, and they will therefore be described in detail.

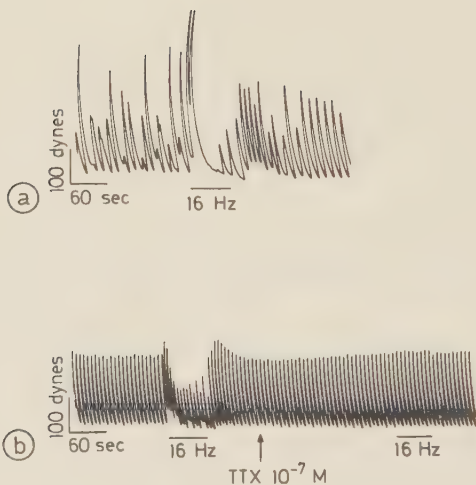


Fig. 1. Isthmus, circular smooth muscle preparation showing spontaneous motor activity. (a) Electrical stimulation of the preparation at 16 Hz induces a twitch contraction that is followed by a rapid reduction in motor activity, after which the spontaneous activity returns. (b) Following administration of tetrodotoxin (TTX), the same stimulation does not reduce the motor activity. Vertical calibration, 100 dynes; horizontal calibration, 60 sec.

Transmural electrical stimulation induced in the circular musculature an almost immediate reduction in tension, often combined with a reduction or abolishment of the spontaneous contractions (Fig 1a). The effect was usually preceded by a "twitch" contraction with an amplitude clearly higher than that of the individual peaks in the spontaneous phasic contractions (13). Following a 1 or 2 min stimulation period the preparation returned to its normal activity in the course of another 1-2 min.

The effect of transmural electrical stimulation, both the "twitch" contraction and the more sustained reduction of the motor activity, was abolished by 10^{-7} - 10^{-6} M tetrodotoxin, which blocks nerve conduction through inhibition of the Na^+ transport across the axonal membrane (Fig 1b). This shows that the response obtained during transmural stimulation is mediated by nerve fibres in the preparations (10). The spontaneous motor activity was not affected.

Administration of β -receptor antagonist, propranolol ($1 - 3 \times 10^{-5}$ M) usually did not inhibit the nerve-induced reduction in motor activity in the circular smooth musculature (Fig 2a). When an inhibition of motor activity did occur, it was only slight. Sometimes the response was even reversed into increased motor activity in the presence of propranolol. When the α -receptor blocking agent, phentolamine ($1 - 3 \times 10^{-5}$ M), was added to the bath containing propranolol there was no change in the response to electrical nerve stimulation compared to the situation with propranolol alone (Fig 2b). In some experiments the preparation was exposed only to phentolamine. Under this condition it was often possible to recognize a slight potentiation of the reduced motor activity obtained by nerve stimulation. The electrically induced inhibition remaining in the presence of 10^{-5} M propranolol and 10^{-5} M phentolamine was resistant also to the adrenergic blocking agent, bretylium (10^{-5} M).

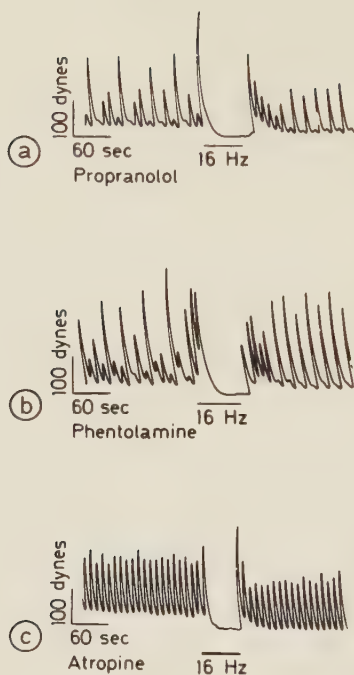


Fig. 2. Recording of circular smooth muscle activity in the isthmus of the tube. (a) The β -receptor antagonist, propranolol ($10^{-5}M$), was given into the organ bath 15 min before electrical stimulation of the nerves in the preparation (16 Hz). The motor response is not effected by the β -antagonist. (b) Addition of the α -receptor blocking agent, phentolamine ($10^{-5}M$), 15 min before the next stimulation at the same frequency was also without effect on the motor response to nerve stimulation. (c) Administration of the cholinergic receptor antagonist, atropine ($10^{-5}M$), 15 min before the electrical field stimulation had no effect on the nerve-mediated motor response. Vertical calibration, 100 dynes; horizontal calibration, 60 sec.

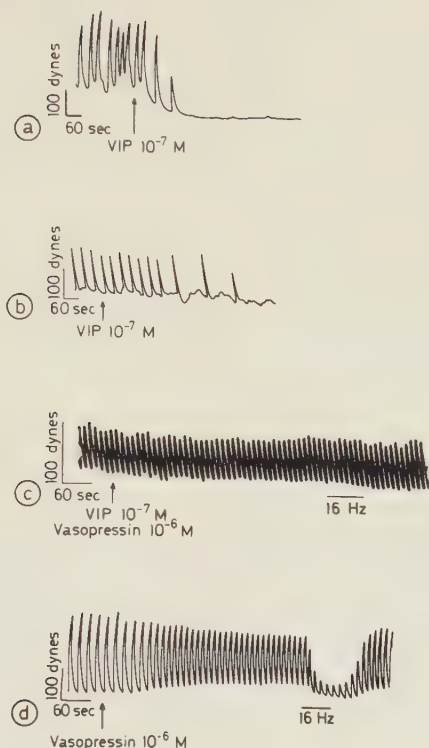


Fig. 3. Effect of VIP on the motor activity in the circularly oriented smooth musculature of the tubal isthmus. (a) In some cases VIP reduced the motor activity with a very short delay. (b) In other cases the same VIP concentration showed a more slowly developing inhibition of tubal motor activity. (c) In the presence of a high concentration of VIP in the bath (together with vasopressin to balance the inhibitory action of exogenous VIP), electrical stimulation of the nerves (16 Hz) had no effect on the motor activity. The bath also contained propranolol, phentolamine and atropine, 10^{-5} M each. (d) Vasopressin alone, which increased motor activity, did not interfere with the nerve-induced tubal relaxation obtained during adrenergic and cholinergic receptor blockade. Vertical calibration, 100 dynes; horizontal calibration, 60 sec.

The cholinergic muscarinic receptor antagonist, atropine (10^{-5} M), given alone or in combination with the previously mentioned adrenergic blocking agents, did not affect the nerve-mediated reduction in motor activity of the circular isthmic smooth muscle (Fig 2c). This confirms the presence of a non-adrenergic, non-cholinergic component in the nerve-mediated inhibition of tubal motor activity (8). Among the first chemical classes considered for non-adrenergic, non-cholinergic transmission were purine nucleosides and nucleotides (4). Unpublished experiments from our laboratory have shown a dose-related relaxation by adenosine and ATP in both circular and longitudinal smooth musculature of the human Fallopian tube, and also cAMP reduces tubal motor activity (7). However, because of the established occurrence of VIP-containing nerves in the human tube, the present study has focused on VIP as a possible candidate for the non-adrenergic, non-cholinergic motor inhibition.

The sequential responses of the longitudinal smooth muscle of the isthmus to the individual antagonists tested were quantitatively less distinct than in the circular muscle and therefore more difficult to interpret. Nevertheless, it was evident that these preparations, in the combined presence of phentolamine, propranolol and atropine in the concentrations given above still responded to transmural electrical stimulation with a reduced motor activity, an effect that was completely abolished by 10^{-6} M tetrodotoxin.

Administration of VIP (10^{-9} to 10^{-7} M) reduced the motor activity of the isthmus (Fig 3a and b), whether the above-mentioned autonomic receptor antagonists were present or not. The effect was more pronounced in the circular than in the longitudinal preparations. The maximum motor inhibition was sometimes achieved within a minute (Fig 3a), but the effect was often slow in onset, reaching a maximum 4-6 min after the administration (Fig 3b). This delay might be due to a slow diffusion of VIP towards the smooth muscle receptors because of diffusion barriers in the preparation. By contrast, the nerve-induced non-adrenergic, non-cholinergic reduction in motor activity was

always rapid in onset and reached a maximum within a shorter period than after VIP administration.

To test the possibility that VIP is responsible for the nerve-mediated reduction in motor activity, transmural electrical stimulation was carried out in the presence of a high concentration of VIP in the bath (10^{-7}M) with the intention to occupy the VIP receptors of the smooth muscle. The reduction in tension induced by VIP was counteracted by vasopressin (10^{-6}M), giving a muscle tension not different from that before adding the drugs. Under these conditions, transmural nerve stimulation no longer reduced the motor activity (Fig 3c). The ability of the tube to respond to VIP (10^{-7}M) was not affected, as tested immediately following rinsing of the preparation. Vasopressin alone (10^{-6}M) caused an increase in tubal motor activity. Electrical field stimulation of the nerves produced the same reduction of the motor activity as before administration of vasopressin (Fig 3d), ruling out that vasopressin acts as inhibitor of the nerve-released relaxatory transmitter.

An experiment was performed in order to test whether the blockade by VIP of the nerve-induced reduction in tubal motor activity was due to specific occupation of the VIP receptors or whether VIP in a nonspecific manner had made the smooth muscle unresponsive to a relaxatory stimulus. As in previous experiments, atropine (10^{-5}M) was used to inhibit any cholinergic response, and bretylium (10^{-5}M) was added to block the adrenergic nerves without interfering with the smooth muscle adrenoceptors. Electrical field stimulation reduced the tubal motor activity in agreement with previous experiments (Fig 4). A combination of vasopressin (10^{-6}M) and VIP (10^{-7}M) abolished the nerve-induced reduction in motor activity, but the preparation still responded with a pronounced reduction in motor activity when $3 \times 10^{-5}\text{M}$ of the β -receptor agonist, terbutaline, was given (Fig 4). This speaks against a nonspecific inhibitory effect of VIP on the nerve-mediated, non-adrenergic, non-cholinergic tubal relaxation.

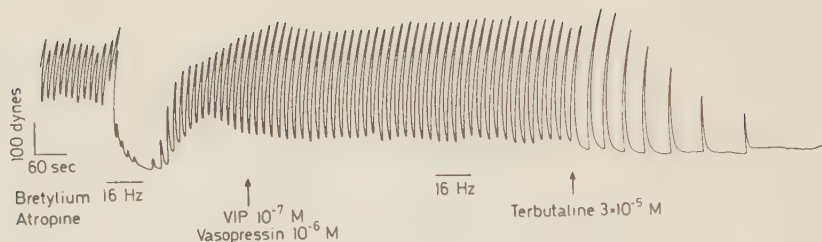


Fig. 4. A combination of VIP and vasopressin abolish the neurogenic non-adrenergic, non-cholinergic reduction of tubal motor activity (induced by 16 Hz nerve stimulation in the presence of 10^{-5} M bretylium and 10^{-5} M atropine). The peptides have not produced a nonspecific desensitization of the tube: reduced motor activity can still be induced by β -adrenergic stimulation with terbutaline. Vertical calibration, 100 dynes; horizontal calibration, 60 sec.

Thus, in conclusion, the isthmic region of the human Fallopian tube is rich in VIP-containing nerves (1). VIP is a potent inhibitor of tubal motor activity (15). Nerve stimulation in this region induces a pronounced inhibition of tubal motor activity that is resistant to adrenergic and cholinergic blockade but abolished by tetrodotoxin. Activation of this non-adrenergic, non-cholinergic neural mechanism in the combined presence of exogenous VIP and vasopressin fails to reduce tubal motor activity. This is to be expected if exogenous VIP and the neurotransmitter released upon electrical stimulation compete for the same receptor. Vasopressin was added to counteract the motor reduction of exogenous VIP. Since inhibition of the motor activity could be elicited in the presence of vasopressin alone, it is unlikely that vasopressin had interfered with the non-adrenergic, non-cholinergic motor inhibition. The results favour the view that VIP is responsible for the neurogenic inhibition of motor activity in the isthmus region in the human Fallopian tube. The VIP fibres may be involved in an efficient opening mechanism in the isthmus of the Fallopian tube, allowing the passage of the fertilized ovum to the site of implantation.

ACKNOWLEDGEMENTS

Supported by the Swedish Medical Research Council (grant No. 14X-5680), the Medical Faculty, University of Lund, and from Maggie Stephens' Foundation for Medical Research, Sweden.

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